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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **352nd Meeting** *of the Biochemical Society was a joint meeting with the Scandinavian Biochemical Societies and was held in the Department of Biochemistry of the University of Cambridge on Thursday, Friday and Saturday,* 28–30 *June* 1956, *starting at* 2 *p.m. on Thursday* 28 *June. The following papers were read:*

COMMUNICATIONS

Cholinesterases in Bovine Plasma. By D. C. HARDWICK. (*Institute of Animal Physiology, Babraham, Cambridgeshire*)

Crude albumin, separated from bovine plasma by the ether technique of Kekwick & Mackay (1954), was found to destroy acetylcholine. As bovine plasma had previously been reported to contain negligible amounts of cholinesterases (Augustinsson, 1948), the cause was further investigated. Using the Warburg technique, bovine plasma was found to hydrolyse acetylcholine and also butyrylcholine. Some separation of the activities into the globulin impurities of the crude albumin could be achieved as Kekwick, Mackay & Martin (1953) had found to be the case for human plasma cholinesterase.

This material hydrolysed acetylcholine and butyrylcholine at approximately equal rates. Acetyl β-methylcholine was attacked at a lower rate and so were triacetin and tributyrin. Benzoylcholine was not attacked. 80–90% of the choline ester and triacetin hydrolysis was inhibited by 10^{-5}M eserine, but only 30–40% of the activity against tributyrin was inhibited; DFP at 10^{-6}M inhibited butyrylcholine hydrolysis; 284C51 (Fulton & Mogey, 1954) at 10^{-6}M inhibited acetylcholine hydrolysis to about 70%. The results suggested the presence of a 'true' (acetyl) cholinesterase and a butyryl cholinesterase; additive experiments with both substrates present failed, since butyrylcholine inhibits true cholinesterase (Cohen, Warringa & Bovens, 1951). A crude separation of the two enzymes has been achieved by ammonium sulphate precipitation.

Recently, Bannister, Whittaker & Wijesundra (1953) found ox spleen to contain a butyryl cholinesterase and Mendel & Myers (1955) found much of this activity was eserine-sensitive. On the basis of inhibition by selective inhibitors the latter authors define this enzyme as a pseudocholinesterase. The butyryl cholinesterase described here may therefore qualify as a 'pseudo' cholinesterase, though it would not be so classified by the earlier definition (Mendel, Mundell & Rudney, 1943) that pseudocholinesterases hydrolyse benzoylcholine. The redefinition indicates the unsatisfactory nature of this nomenclature.

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The Metabolism of Azo-dyestuffs. I. The Metabolism of Benzeneazo-2-naphthol. By J. W. DANIEL. (*Industrial Hygiene Research Laboratories, Imperial Chemical Industries Limited, The Frythe, Welwyn, Herts*)

Although studies have been made of the toxicology of azo-compounds little is known of the chemical changes undergone by them in the tissues. Special interest attaches to those azo-dyestuffs which find application as food colouring agents.

Cleavage of the azo-link within the tissues has been demonstrated for a number of azo-compounds. Sisley & Porcher (1911) identified sulphanilic acid in the urine of dogs dosed with *p*-sulphobenzeneazo-1-naphthol (Orange 1); Fuller (1937) isolated

sulphanilamide from the urine of patients who had been treated with soluble prontosil; cleavage of the carcinogen *p*-dimethylaminoazobenzene has been demonstrated by Stevenson, Dobriner & Rhoads (1942). Elson & Warren (1944) found *p*-aminophenol to be a urinary metabolite of azobenzene.

Benzeneazo-2-naphthol (0·5 g./kg.) was administered to rabbits by stomach tube. Descending chromatography was used to identify the principal urinary metabolites. These were found to be *p*-hydroxybenzeneazo-2-naphthol, *p*-hydroxyacetanilide, 1-amino-2-naphthol, *p*-aminophenyl glucuronide, 1-amino-2-naphthyl glucuronide and 1-amino-2-naphthyl sulphate. Aniline was also identified in the steam volatile distillate of the alkaline urine (pH 10).

Fifty-one per cent. of the dose of benzeneazo-2-naphthol could be accounted for as urinary metabolites. Fission products accounted for 85% of the benzeneazo-2-naphthol excreted.

The absence of *o*-aminophenol suggests that hydroxylation occurs prior to fission of the azo-compound. Whether the aniline present is to be considered a urinary artifact or whether it results from the failure of a part of the benzeneazo-2-naphthol to be hydroxylated is not clear. Retention of material would appear to be high, and there is evidence to show that the compound is retained in the tissues in modified form.

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The Detection and Estimation of Plasma Albumin in Rat and Rabbit Skins. By J. H. Humphrey, A. Neuberger and D. J. Perkins. (*The National Institute for Medical Research, Mill Hill, London, N.W. 7, and Department of Chemical Pathology, St Mary's Hospital Medical School, London, W. 2*)

Evidence for the presence of plasma proteins in the skins of young rabbits has been previously obtained by electrophoretic analysis of a non-collagenous soluble protein extract (Harkness, Marko, Muir & Neuberger, 1954). We have confirmed this and made further studies on the rate of distribution of intravenously injected plasma proteins into the skin of adult rabbits.

Adult animals were bled, killed and skinned. The skins were frozen, sliced and minced before extraction three times with M/15, pH 8·0, phosphate buffer. The extracts were exhaustively dialysed against distilled water. Insoluble material was discarded before freeze-drying the protein solution. The freeze-dried product was suspended in the pH 8·0 phosphate buffer and insoluble material discarded. Globulins were removed by making the solution 26% with respect to sodium sulphate. The supernatant was dialysed free of salt and freeze-dried.

The plasma albumin was characterized by electrophoresis, immunology, ultraviolet spectrum and the optical rotation in both guanidine-denatured and native states.

Further experiments using intravenously injected ^{131}I- and ^{14}C-labelled homologous plasma albumin showed that equilibrium between the vascular and skin compartments was slower than the apparent overall equilibrium for whole animals observed by Cohen, Holloway, Matthews & McFarlane (1956).

Quantitative estimates of the plasma albumin in skin showed that at equilibrium there is albumin equivalent to at least 12 ml. of serum for each 100 g. of wet skin. This, in terms of protein, is equivalent to 0·5% of the wet weight of skin. The specific activity of ^{131}I-labelled albumin from skin even 20 days after injection is only 60% of that circulating in the plasma. The remaining 40% of activity cannot be explained by impurities which are at most 10% of the total. Possible explanations are either incomplete equilibration, or the presence of some non-exchangeable albumin in skin.

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The Demonstration of Acid Mucopolysaccharides in the Glands of Lorenzini of the Piked Dogfish (*Acanthias vulgaris*). By C. E. JENSEN (introduced by the COMMITTEE). (*Institute of Physical Chemistry, University of Copenhagen, Denmark*)

It has previously been found (Jensen, 1954; Vilstrup & Jensen, 1954) that the endolymph of Elasmobranchii contains hyaluronic acid. As the inner ear and the Lorenziniic glands are considered to have the same ectodermal origin it could be expected that hyaluronic acid was present in these structures too.

The glands containing their mucinous secretions were homogenized, and extracted by a procedure similar to that described for umbilical cords (Jensen, 1953). A greyish-white powder was obtained, after purification and desiccation of the precipitate formed on adding alcohol to the aqueous extract. This powder yielded a highly viscous solution when dissolved in water at a concentration of about 0·1 %.

The viscosity of this solution decreased very markedly when it was incubated with a commercial sample of testicular hyaluronidase, the relative viscosity approaching 1 with time. A distinct decrease in viscosity was also observed on incubation with a sample of hyaluronidase prepared from haemolytic streptococci. However, the final value of the relative viscosity was much greater than that obtained from the experiment in which the testicular enzyme had been employed, in spite of repeated additions of bacterial enzyme. But after the addition of testicular hyaluronidase decrease in viscosity again occurred, the final value being only a little higher than 1.

As pointed out by Meyer & Rapport (1951) testicular hyaluronidase hydrolyses hyaluronic acid as well as the chondroitin sulphates A and C, whereas hyaluronidases of bacterial origin attack hyaluronic acid only. Therefore it may be concluded that the Lorenziniic glands contain both hyaluronic acid and chondroitin sulphates.

A solution of the isolated material was incubated with streptococcal hyaluronidase under such conditions that it was to be expected that the hyaluronic acid present in the preparation had been degraded. Then the solution was dialysed against water for 5 days at 4°. After the evaporation of water from the dialysed solution, measurements of the osmotic pressure were performed on solutions of the residual material using the technique described by Christiansen & Jensen (1953). Based upon these measurements the molecular weight of the substance was estimated at 98000.

Both the glandular tissue and solutions of the isolated substances were metachromatic with toluidine blue.

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The Metabolism of Acetaldehyde in Rat Liver Homogenates. By F. LUNDQUIST and E. KLÄNING. (*University Institute of Forensic Medicine, Copenhagen*)

The mechanism of acetaldehyde removal in liver is of considerable interest as this substance is an intermediate in the oxidation of ethanol, which may furnish a large part of the energy requirement in the animal body. Several enzymes may be involved in the metabolism of acetaldehyde, including DPN-linked aldehyde dehydrogenase and alcohol dehydrogenase, the flavin enzyme aldehyde oxidase, and possibly the enzyme systems leading to deoxyribose phosphate and acetoin.

An approach to the problem of the relative importance of these various pathways was made by measuring aldehyde disappearance from liver slices and crude liver homogenates. Determinations of acetaldehyde in deproteinized samples were made by means of an enzymic method involving the use of aldehyde dehydrogenase from yeast (Black, 1951). This enzyme as an analytical tool has the advantage over the corresponding enzyme from liver (Racker, 1949) that it does not contain any DPN-requiring lactic dehydrogenase. The velocity of the reduction of DPN by the yeast enzyme is proportional to the concentration of acetaldehyde within wide limits.

Homogenates (1:10) of rat liver in 0·15M-KCl containing 0·05M phosphate, pH 7·0, display a rate of aldehyde disappearance under anaerobic conditions at 21° of 20–30 μg./min./g. liver. The rate is increased some 3 times when DPN is added in amounts corresponding to the concentration in rat liver (Glock & McLean, 1955). Further increase in DPN concentration does not augment the rate of

aldehyde disappearance. Variations in the initial concentration of acetaldehyde from 5 to 50 μg./ml. had no influence on the reaction rate.

When similar experiments are made under aerobic conditions, but without DPN addition, acetaldehyde is metabolized somewhat faster. The increase corresponds to about 10 μg. acetaldehyde/min./g. liver. This increase is presumably caused mainly by flavin enzymes, as addition of 0·001M-NaCN does not influence the reaction.

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Enzymic Conversion of Acetylenic Bonds. By K. E. Eimhjellen (introduced by the Committee).
(*Department of Biochemistry, The Technical University of Norway, Trondheim, Norway*)

In the present investigation several strains of bacteria have been isolated in pure culture from soil and sewage on a medium containing acetylene dicarboxylic acid (ADA), butyne-1:4-diol, or propynol as the only sources of carbon. One strain, shown to belong to the family of Enterobacteriaceae, was chosen for studies on the enzymic conversion of ADA. In a previous communication evidence was presented that oxaloacetate is an intermediate in the anaerobic dissimilation of ADA by resting cells of this bacterium (Eimhjellen, 1956).

Cell-free extracts are obtained by crushing thick suspensions of bacteria in the Hughes press at $-20°$ and extracting the paste with water or 0·006M-NaF.

Extracts from aerobically grown cells convert ADA anerobically to oxaloacetate, which is further decomposed slowly to pyruvate and CO_2. The optimum pH for the production of oxaloacetate is about 7·5. The Michaelis constant is 0·008M.

In extracts from anaerobically grown cells the same oxo-acids accumulate from ADA, but in lower yields. A concomitant high CO_2 production indicates a further conversion of the oxo-acids to products characteristic of an enterobacter fermentation. The extracts keep most of their activity towards ADA for 1–2 months at $-20°$. The work described below was done with crude extracts of aerobically grown cells.

Dialysis for 2–4 hr. against 0·01M phosphate buffer (pH 6·8) or water reduces the activity 10–50%. Activity is completely lost after overnight dialysis and is not restored by boiled preparations of the cell-free extract, nor by cysteine, reduced glutathione, DPN, ATP, Mg^{2+}, CoA or combinations of these compounds. The conversion of ADA is 75% inhibited by 0·0028M-NaF; the activity is nearly completely restored by the addition of 0·03M-Mg^{2+}. Nevertheless, 0·001–0·01M-NaF greatly stabilized the enzyme activity. The crude extracts contain fumarase and malic dehydrogenase. The latter enzyme is inactivated by heating small volumes of the extract anaerobically for 1 min. in a water-bath at 70° without appreciable loss of ADA-decomposing activity. This observation strongly suggests that oxaloacetate is formed from ADA by direct hydration of the triple bond and excludes a pathway consisting of a hydrogenation of ADA to fumarate followed by conversion to oxaloacetate by well-known enzyme reactions. The observed fluoride sensitivity of the enzyme system converting ADA to oxaloacetate indicates that oxaloacetate is first produced as the enol form which is then enzymically converted to the oxo form.

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Benzimidazole Derivatives and Glycine Incorporation in Disrupted Staphylococcal Cells. By E. F. Gale and Joan P. Folkes. (*Medical Research Council Unit for Chemical Microbiology, Department of Biochemistry, University of Cambridge*)

In an attempt to throw further light on the role of nucleic acids, and 'incorporation factors' derived therefrom, in the incorporation of amino-acids, a survey has been made of the actions of purine and pyrimidine analogues on glycine incorporation in disrupted staphylococcal cells. Of the substances so far tested, derivatives of benzimidazole have proved to possess the most interesting properties.

Benzimidazole is a weak inhibitor whose activity is markedly modified by substitution in positions 1 and 6. 0·001M 5:6-Dimethylbenzimidazole produces approximately 80% inhibition; substitution of an allyl-group in position 1 increases the inhibitory action by some 50 times, whereas similar substitution of a glucopyranosyl- or 2:3-dihydroxypropyl- group abolishes the inhibitory action. The natural dimethylbenzimidazole ribotide has an inhibitory action of the same order as that of the base alone. Benzotriazole and 5:6-dimethylbenzotriazole are without effect; 4-methoxy-6-nitrobenzotriazole is a potent inhibitor.

Substitution of an amino-group in position 6 produces derivatives which stimulate glycine incorporation; the most effective stimulators so far found being 6-amino-4-hydroxybenzimidazole or 6-amino-4-hydroxybenzotriazole. The inhibitory benzimidazole derivatives are antagonized by the stimulatory derivatives. Thus inhibition by 5:6-dimethylbenzimidazole is completely antagonized by equimolar concentrations of 6-amino-4-hydroxybenzotriazole, whereas guanine or adenine are much less effective antagonists.

Possible implications of these findings will be discussed.

Ethylenediaminetetra-acetate and the Adenosinetriphosphatase Activity of Actomyosin Systems. By S. V. Perry and T. C. Grey. (*Department of Biochemistry, University of Cambridge*)

At pH 7·4 in the presence of 5 mM-$MgCl_2$ and 5 mM-ATP the adenosinetriphosphatase (ATPase) activity of myofibrils and 'natural' actomyosin prepared from rabbit skeletal muscle was reduced by 0·2–0·5 mM ethylenediaminetetra-acetate (EDTA) to a level below that obtained in the absence of $MgCl_2$. This chelating agent has a greater affinity for Ca^{2+} ions than Mg^{2+} ions (Schwarzenbach & Ackermann, 1947); nevertheless, with $CaCl_2$ as activator (or with $MnCl_2$) appreciable inhibition of the myofibrillar ATPase was only obtained when approximately equal concentrations of these activators and EDTA were present. Similar effects were also obtained with ethyleneglycol-bis-β-amino-ethylether-*N*, *N'*-tetra-acetate ('glycolkomplexon': Senn, 1954; Raaflaub, 1955).

Repeated precipitation by dilution of 'natural' actomyosin extracted from isolated myofibrils and from whole muscle did not significantly modify the effect of EDTA on the magnesium-activated ATPase (Mg-ATPase), whereas the Mg-ATPase of 'synthetic' actomyosin prepared by combining purified F-actin and L-myosin was not sensitive to low EDTA concentrations. In general the action of EDTA on the magnesium- and calcium-activated ATPase of 'synthetic' actomyosin and on the calcium-activated ATPase of 'natural' actomyosin were similar. Likewise the Mg-ATPase of aqueous extracts of acetone powders of rat liver mitochondria was not sensitive to low concentrations of EDTA.

From the point of view of relaxation studies these findings suggest that the relaxing action of EDTA (Bozler, 1954; Watanabe, 1955) is due to inhibition of the Mg-ATPase of the myofibril. The extreme sensitivity of the system to EDTA indicates that the mechanism of ATP hydrolysis by myofibrils and 'natural' actomyosin in the presence of magnesium possesses certain unusual features which are not apparent in the 'synthetic' system studied under similar conditions. One possible implication is that traces of an unidentified metal bound to 'natural' actomyosin (cf. Friess, Morales & Bowen, 1954), are essential for the Mg-ATPase. If such traces of metal are responsible for the effects described they are not removed by isolating myofibrils in media containing EDTA; furthermore, after treatment with EDTA followed by repeated washing of the myofibrils the Mg-ATPase activity is largely restored and can be inhibited once more by low concentrations of EDTA.

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Inhibition by Propionate of Acetate Metabolism in Liver Slices. By R. J. PENNINGTON. (*Rowett Research Institute, Bucksburn, Aberdeenshire*)

A number of instances have been reported of inhibitory effects of propionic acid upon animal tissues or micro-organisms (Grafflin & Green, 1948; Hill, 1952; Lang & Bässler, 1953; Pennington, 1954). In most cases, this has been attributed to combination of propionate with coenzyme A.

In the presence of an equimolar amount of propionate the amount of $^{14}CO_2$ formed from [*carboxy*-^{14}C]acetate by rat liver slices was decreased to usually less than 5%. The total quantity of acetate disappearing, and the amount of ^{14}C found in the acetone derived from the ketone bodies formed, were lowered to a similar degree. The rate of oxidation of [*carboxy*-^{14}C]propionate was not decreased by acetate.

The formation of $^{14}CO_2$ from [*carboxy*-^{14}C]acetate by rat kidney, heart or diaphragm was decreased by propionate, but the effects were much smaller than obtained with liver.

Butyrate, *iso*-valerate, valine, isoleucine, and methionine decreased, to varying degrees, the oxidation of acetate by liver slices.

Propionate did not markedly affect the oxidation of [*carboxy*-^{14}C]butyrate. The oxidation of [2-^{14}C]pyruvate was decreased to about one-half by propionate.

Similar experiments with sheep liver slices showed a marked species difference in the magnitude of the effect of propionate. The uptake and oxidation of acetate in the presence of propionate were usually over 50% of the values obtained without propionate. Endogenous ketone body production by sheep liver was much less than with rat liver.

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Improved Methods for the Preparation of Comirin from Cultures of *Pseudomonas antimycetica*. By J. J. GORDON, B. K. KELLY and G. A. MILLER. (*Medical Research Council Antibiotics Research Station, Clevedon, Somerset*)

Forsyth (1955) described methods for the assay and isolation of comirin, an antifungal antibiotic obtainable from cultures of a species of bacterium related to *Pseudomonas fluorescens*, first described by Thaysen & Thaysen (1953). Using a peptone-sucrose medium, Forsyth was able to obtain cultures of approximately 25 units/ml. activity (equivalent to 25 μg./ml. of standard preparation).

Our investigation of the culture conditions of this organism has revealed that, under optimal conditions of aeration and stirring, antibiotic titres of over 1000 units/ml. are obtainable on a medium containing soya meal as nitrogen source.

It was then necessary to modify the standard extraction procedure, which, though satisfactory when applied to cultures of the levels of activity reported by Forsyth (1955), gave inadequate recoveries in the high-activity cultures. Initial heating to 100° at pH 3, and centrifuging in the hot through a Sharples centrifuge, resulted in the removal of a large amount of inert material with only slight loss of activity. The supernatant was then adjusted to pH 6·5 and extracted several times with *n*-butanol at 70°. From that stage on, the Forsyth procedure was followed.

With the above improvements in fermentation and extraction, comirin could be obtained in yields of 33·5 million units per 100 l. culture, compared with 1·4 million units per 100 l. normally obtainable by the original procedure.

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The Incorporation of [^{14}C]Formate into the Nucleic Acids of the Ehrlich Ascites Carcinoma of the Mouse. By R. Y. Thomson, R. M. S. Smellie, R. Goutier and J. N. Davidson. (*Department of Biochemistry, The University of Glasgow*)

In vivo, the Ehrlich ascites carcinoma of the mouse incorporates [^{14}C]formate into the purines of deoxyribonucleic acid (DNA), nuclear ribonucleic acid (nRNA) and cytoplasmic ribonucleic acid (cRNA), and into the thymine of DNA. Incorporation into nucleic acid pyrimidines other than thymine is negligible. If the ascites cells and plasma are removed from the peritoneal cavity and incubated with ^{14}C-formate in roller tubes the incorporation into the purines of DNA and cRNA is virtually abolished and that into the purines of nRNA greatly reduced, although incorporation into DNA thymine remains high. This is not due to cessation of the metabolic activities of the cells since both [8-^{14}C]adenine and inorganic $^{32}PO_4^{3-}$ are well incorporated into DNA, nRNA and cRNA *in vitro*. Formate incorporation into DNA thymine is depressed by incubation in an atmosphere of nitrogen and stimulated by addition of ATP. This compound has, however, no effect on incorporation into nucleic acid purines.

Good incorporation of formate into nucleic acid purines can be obtained *in vitro* by addition of an extract of pigeon liver, mouse liver, mouse spleen or mouse skeletal muscle prepared by homogenizing the tissue in the incubation medium described by Schulman, Sonne & Buchanan (1952) and centrifuging the homogenate at 100000 *g* for 40 min. to remove cell particles. Dialysis of the mouse liver extract for 48 hr. against the same medium appears to reduce, but not to abolish, its capacity for stimulating incorporation into nucleic acid purines.

Rabbit bone marrow suspended in Krebs–Ringer bicarbonate buffer also incorporates [^{14}C]-formate into the nucleic acid purines to a much lesser extent than *in vivo*, although incorporation into DNA thymine is of the same order in both cases. As in mouse ascites tumour, incorporation into nucleic acid purines *in vitro* is stimulated by addition of liver extract.

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The Metabolism of Sulphated Polysaccharides in Carrageenin-induced Granuloma. By H. G. B. Slack. (*Rheumatism Research Centre, University of Manchester*)

As part of a series of biochemical and histological investigations into connective tissue granuloma induced by carrageenin injection (Jackson, 1956), the metabolism of sulphated polysaccharides has been studied. Carrageenin has been found to produce an excellent experimental model for studying active connective tissue formation and breakdown.

Carrier-free $Na_2{}^{35}SO_4$ (1·4 μc/g. body weight) was injected intraperitoneally into guinea pigs 5 days after carrageenin, injected subcutaneously. The animals were killed at various time intervals afterwards and organically-bound sulphate isolated in three fractions; a salt-soluble fraction extracted with 0·2M-NaCl, pH 7·4, a papain fraction at pH 6·0, and residue sulphate which resists these two preceding extractions.

The sulphated polygalactose, carrageenin, in the extracts was selectively removed by precipitation with cetylpyridinium chloride in 1·5M-KCl (Scott, 1956). Sulphate determinations were done by a modified method of Dodgson & Spencer (1953), and radioactivities measured as $BaSO_4$ in infinite thickness with known amounts of carrier $BaSO_4$.

Salt-soluble polysaccharide sulphate is already high 3–5 days after carrageenin, falls rapidly for the next 7 days and then more slowly. Papain fraction sulphate is highest 6 days after carrageenin, falls rapidly for the next 3–4 days and then more slowly. Residue sulphate is smaller in amount and shows no large variations.

The specific activity of the salt fraction is highest at 10 hr., falling rapidly, the estimated 'half-life' being 2 days. Specific activity is maximum in the papain fraction at about 30 hr. and the estimated 'half-life' 7 days. Residue sulphate shows low specific activities, decreasing slowly with time. The specific activity/time curve for the salt-soluble fraction is similar to the neutral collagen, whereas the papain fraction seems more related to the acid and insoluble collagens. Residue sulphate appears independent of the chain of metabolic events in this highly active connective tissue.

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The Formation and Removal of Collagen in the Carrageenin Granuloma. By D. S. JACKSON. (*Rheumatism Research Centre, University of Manchester*)

It has been reported previously (Robertson & Schwartz, 1953; Jackson, 1956) that carrageenin injected subcutaneously into guinea pigs stimulates the formation of connective tissue. The formation of three collagen fractions, viz. neutral salt-soluble collagen (N.C.), acid-soluble collagen (A.C.) and insoluble collagen (I.C.), in this situation was studied (Jackson, 1956). The present communication extends the time of observation from 14 days to 28 days and also reports the incorporation of [^{14}C]glycine into these collagen fractions.

The methods have been previously described (Jackson, 1956). In addition, [^{14}C]glycine (10 μc/100 g. body weight) was injected intraperitoneally 5 days after carrageenin injection, and the animals killed 2, 6, 8, 12, 24, 48 hr. and 4, 7, 14 or 28 days afterwards. Samples of the three collagen fractions were purified, hydrolysed and DNP-glycine isolated from them by the method of Perrone (1951). Radioactivities were measured on solid samples at 'infinite thinness' by the method of Henriques, Henriques & Neuberger (1955).

Maximum concentration of collagen is reached after 14 days, but the wet weight of tissue reaches a maximum at about 9 days and falls rapidly thereafter, the tissue disappearing after 4–5 weeks. The decrease in net weight is accompanied by a considerable increase in N.C. and A.C., suggesting that during break-down I.C. is first transformed into the soluble types of collagen.

[^{14}C]Glycine is incorporated most rapidly into N.C., maximum incorporation occurring after about 10 hr., with a rapid fall within 24 hr. There is a slower incorporation into A.C. and I.C., maximum incorporation occurring at about 12 and 24 hr. respectively, that for I.C. being greater than that for A.C.

These results confirm the suggestion made by Harkness, Marko, Muir & Neuberger (1954) that (*a*) N.C. is the true precursor of mature collagen, and (*b*) that A.C. need not be an intermediate between N.C. and I.C.

It is suggested that neutral collagen can be handled in two ways: (*a*) to form new fibrils which behave like A.C. (cf. Jackson & Fessler, 1955), which accumulates further N.C. to form I.C.; and (*b*) is incorporated directly into I.C., increasing the thickness of the pre-formed insoluble fibres.

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The Formation of [^{14}C]Hydroxyproline from L[^{14}C]-Proline by Collagen-forming Cells *in vitro*. By R. H. SMITH and S. FITTON JACKSON. (*Medical Research Council Biophysics Research Unit, Wheatstone Laboratory, King's College, London, W.C. 2*)

Feeding experiments led Stetten & Schoenheimer (1944) to conclude that a significant part of the isotopically-labelled L-proline administered to intact rats was converted to hydroxyproline which accumulated in the carcass protein. The experiments to be described were devised to study the ability of collagen-forming tissue to hydroxylate L-proline *in vitro*.

Osteoblasts were grown in tissue culture from a cell suspension obtained from embryonic chick frontal bone in a fluid medium prepared from a mixture of embryo extract, plasma and Tyrode solution. After 1–5 days' growth the medium was replaced by a similar one containing 0·5 μc L[^{14}C]-proline per ml. The cultures were harvested 1 or 2 days after this addition. Free ^{14}C-amino acids were removed by trichloacetic acid and alcohol-ether treatment; the whole cultures were then hydrolysed (5N-HCl at 100° for 24 hr.) and the amino acids separated by 2-way paper chromatography. A measure of the conversion of proline to hydroxyproline was obtained by comparing the radioactivity of the hydroxyproline spot with that of proline; the figures given are the activity of the hydroxyproline expressed as a percentage of that of proline.

The amount of hydroxyproline formed during the 24 hr. exposure to L[^{14}C]-proline appears to vary with the age of the culture and the corresponding development of extracellular material; 24 hr. cultures give 1% or less conversion and 5-day cultures approximately 5%. Approximately twice

as much radioactivity was found in the glutamic acid as in the hydroxyproline separated from these cultures. Osteoblast cultures grown similarly in the presence of L[^{14}C]-glutamic acid gave proline and hydroxyproline spots which were not radioactive.

Conversion of L[^{14}C]-proline to hydroxyproline has also been obtained with fibroblast preparations obtained from guinea pigs by the subcutaneous injection of 1% solution of carrageenin (Robertson & Schwartz, 1953). Either excised pieces of fibroblast-rich tissue or homogenates thereof were incubated aerobically at 37°, with shaking, in a medium containing K^+, Mg^{2+}, PO_4^{3-}, Cl^-, ATP, succinate and L[^{14}C]-proline. After prolonged incubation a significant amount of radioactivity (up to 6% of the incorporated proline activity) was shown in the hydroxyproline fraction obtained from the tissue.

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Invertebrate Tropomyosin. By K. Bailey. (*Department of Biochemistry, University of Cambridge, and Stazione Zoologica, Naples*)

The adductor muscles of many lamellibranchs contain a component or components which give characteristic X-ray diffraction and electron-optical patterns quite distinct from those of mammalian skeletal muscle (Bear, 1944; Hall, Jakus & Schmitt, 1945). The fibrils responsible for this pattern are especially abundant in the slow, smooth parts of adductor muscles, and were named by Schmitt's group Type I fibrils or paramyosin. An attempt has been made to characterize paramyosin chemically by studying the entirely smooth adductor of *Pinna nobilis* and both smooth and striated portions of oyster adductor. Extracts of smooth muscle at pH 7 and ionic strength $>0{\cdot}5$, though very viscous, respond only feebly to tests characteristic of myosin or of actomyosin. On dialysis to $I=0{\cdot}3$, the viscous component crystallizes in birefringent needles, the yield being as high as 30% of the total muscle protein (Bailey, 1956).

In the case of unstriated adductors, crystals can readily be prepared from salt extracts either of fresh muscle or of muscle dehydrated with ethanol-ether, as in the procedure for rabbit tropomyosin (Bailey, 1948). Only the latter method, however, is effective in the case of striated adductors.

Amino acid analysis by the Moore and Stein method on *Pinna* protein (kindly performed by Miss B. D. Warboys) shows that the protein is a tropomyosin. Like rabbit tropomyosin (cf. Bailey, 1948, 1954; Kominz, Hough, Symonds & Laki, 1954), it contains no tryptophan or proline, and the number of residues/10^5 g. with respect to phenylalanine, glycine (both characteristically low), methionine, tyrosine, alanine, valine, leucine, isoleucine, serine, threonine and histidine are the same as for rabbit protein; but arginine residues appear to have replaced part of the lysine, aspartic some of the glutamic, and the amide N is much higher. In these differences may reside the greater insolubility of invertebrate tropomyosin.

The intrinsic viscosity and flow birefringence indicate that the protein is even more asymmetric than rabbit *myosin*. X-ray investigations by Dr E. Beighton (unpublished) on oriented films have yielded a well-defined α-pattern, but only one meridional spacing (70 Å.) characteristic of paramyosin. The contribution of the protein to the pattern typical of Type I fibrils is thus still obscure. The protein is not confined to adductors: similar crystals have been obtained in good yield from the arm muscle of octopus.

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The Chemical Composition of Earthworm Cuticle. By M. R. Watson and R. H. Smith. (*Medical Research Council Biophysics Research Unit, Wheatstone Laboratory, King's College, London, W.C. 2*)

The wide-angle X-ray diffraction pattern of earthworm cuticle is indistinguishable from that given by the fibrous protein collagen, derived from connective tissues of vertebrate animals (Reed & Rudall, 1948; Rudall, 1950). Chemical analyses have been performed upon earthworm cuticle in order to determine whether there are also similarities in composition between the cuticle and collagen.

Earthworms (*Lumbricus* sp.) are immersed in ether, and after 15 min. the cuticles may be easily removed by stripping back from the head with forceps. The cuticles are then washed in numerous changes of distilled water to remove adherent epidermal cells.

It has been found that reproducible analytical results may be obtained with different preparations of cuticle. The cuticle (N, 14·6%) contains about 80% by weight of protein, the remainder being chiefly non-nitrogenous polysaccharide.

Amino acid analysis of the protein component has been carried out by the method of Moore & Stein (1951). The cuticle resembles the vertebrate collagens in having a high glycine content (24·6). (All values given in g. amino acid N/100 g. cuticle nitrogen.) The amino acids with non-polar side-chains all have values similar to those found in collagen. Thus the chemical relationship between the proteins is clearly defined, and this is confirmed by the high hydroxyproline (12·6) content of the cuticle, an imino acid not found in large quantities in any other proteins except the collagens. In fact, earthworm cutcle contains a higher proportion of hydroxyprolyl residues than any collagen yet analysed.

The main differences in composition between the cuticle and vertebrate collagen may be summarized as follows: in parallel with the increased hydroxyproline content, the hydroxyamino acids serine (8·1) and threonine (5·0) are higher in the cuticle, while the imino acid proline (1·2) is present to only a small extent. The basic amino acids, lysine (2·5) and arginine (6·5), are less abundant in the cuticle; the balance of charge in the protein appears to be maintained by a large number of carboxyl side chains being present in the amide form.

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The Steryl Ester and Sterol Fractions of Rat Livers. By J. B. M. Rattray, R. P. Cook and A. T. James. (*Department of Biochemistry, Queen's College, Dundee* (*University of St Andrews*), *and National Institute for Medical Research, Mill Hill, London, N.W. 7*)

The general composition of the lipid extract (ether- and ethanol-extractable material) of the livers of rats fed diets containing olive oil and cholesterol was reported by Rattray & Cook (1956). Further investigations of the acids present in the major sterol-containing fraction (24% of the total extract and containing 66% of the total extracted sterol) have been made by gas-liquid chromatography (James & Martin, 1956). The composition of the acids is (as percentage of total determined acids): linoleic 56·8; oleic 29·5; palmitic 6·3; palmitoleic 2·2. Small amounts of *n*- and unsaturated and/or branched-chain acids from C_6 to C_{19} were found. Of the initial acids 3% were undetermined (i.e. greater than C_{19}). The results are similar to those of Kelsey & Longenecker (1941). Investigation of glyceride fractions shows that linoleic is mainly associated with sterol. A smaller variety of fatty acids is present in sterol fractions than in the glycerides.

The total sterol (digitonin-precipitable material) present was separated into stanols and stenols via the dibromide. The stenols purified by the oxalic acid adduct (cf. Schwenk, Todd & Fish, 1954), gave approximately one-quarter of their weight of reasonably pure Δ^5-stenol ('cholesterol').

Evidence as to the nature of the remaining stenols and to the acids present in the steryl esters of fractions of a less and of a more polar nature will be presented.

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Interrelationships between Diaminopimelic Acid, Lysine and their Analogues in Mutants of *Escherichia coli*. By PAULINE MEADOW and ELIZABETH WORK. (*University College Hospital Medical School, London, W.C.* 1)

Escherichia coli 173–25 (Davis, 1952) requires *meso*- or LL-diaminopimelic acid (DAP) and lysine for growth in a salts-glucose medium (Hoare & Work, 1955*a*). This strain, a second mutant D requiring DAP only, a lysine-requiring mutant 26–26, and the parent strain 9637, have been studied in liquid culture.

DD-DAP, cystine and several straight-chain diaminodicarboxylic acids did not act as growth factors. Lanthionine and cystathionine, reported to support the growth of 173–25 (Seibert, Soto-Figueroa, Miller & Seibert, 1954), were equally ineffective. In the absence of lysine, lanthionine and cystine inhibited growth competitively. 173–25 and D had more active DAP decarboxylases and DAP racemases (Hoare & Work, 1955*b*) than the parent strain.

For 173–25 and 26–26, lysine was replaceable by α-acetyllysine but not by ω-acetyllysine. For rats, the latter replaced lysine, while α-acetyllysine was ineffective (Neuberger & Sanger, 1943). Hydroxyaminocaproic acid, a lysine antagonist in rats (Gaudrey, 1954), had no effect on any mutant. The amount of polylysines required to inhibit growth of 173–25 and D was at least 100 times less than the inhibitory concentration for 9637 (cf. Katchalski *et al.*, 1953). Cadaverine was without effect.

Growth of 173–25 and D was followed turbidimetrically. In the presence of lysine (L or D) the logarithmic (5–12 hr.) growth rate, was independent of DAP concentration. At concentrations of 2×10^{-5}M-DAP or less, turbidity decreased logarithmically 6–9 hr. after inoculation; the time at which lysis began varied with DAP concentration (Hoare, unpublished). In the absence of lysine, D did not lyse. Lysis was unaffected by 100-fold variations in the concentrations of lysine, glucose and phosphate, unlike the lysis of *Streptococcus faecalis* (Toennies & Gallant, 1949). Lysis of 173–25 and D was prevented by replacing lysine by α-acetyllysine or adding lanthionine but not by adding α- or ω-acetyllysine, cystine, cadaverine or hydroxyaminocaproic acid. In the presence of lysine, subinhibitory concentrations of polylysines caused an increased lag period and less total growth but did not prevent lysis.

The fact that these DAP-requiring mutants lysed only in the presence of lysine and not with α-acetyllysine, suggests some antagonism between DAP and lysine utilization. The effects of lanthionine suggest that it might inhibit some reaction which without lysine is essential for growth, but with lysine causes lysis.

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On Sialic Acids and Their Quantitative Estimation. By L. SVENNERHOLM (introduced by the COMMITTEE). (*Department of Medical Biochemistry, University of Gothenburg, Gothenburg, Sweden*)

A method has been devised for the isolation of sialic acids from tissues. (The name sialic acid has been proposed for the non-acylated acid (Svennerholm, 1956*a*).)

The materials were freed from cations and organic substances of low molecular weight by washing with weak sulphuric acid at 0°. Sialic acid was liberated with 0·03N sulphuric acid at 80° and purified by chromatography on ion-exchange resins. On crystallization of sialic acid from dry methanol, partial methyl ester formation occurred, which could be indicated by partition chromatography. Only *N*-acetylsialic acid was found in human material. X-ray diffraction pattern and infrared absorption curve were identical with those of ovine sialic acid (Blix, Lindberg, Odin & Werner, 1955). Potentiometric titration gave a pK of 2·6 and a molecular weight of 308 (calc. for $C_{11}H_{19}NO_9$; 309·3). 2-Carboxypyrrole was formed, when *N*-acetylsialic acid or its methyl ester were heated with 0·08N sodium hydroxide at 100° for 1 hr.

The optimal conditions for the determination of sialic acid in tissues have been investigated. The following colorimetric methods were used: direct Ehrlich reaction, diphenylamine reaction (Dische, 1930), Bial's reaction and a resorcinol method (Svennerholm, unpublished). In the resorcinol method aliquots of sample and reagent (0·2% resorcinol in 30% hydrochloric acid $+Cu^{2+}$) were

heated on a boiling-water bath for 15 min. The colour was extracted into amyl alcohol and the optical density at 580 mμ. determined in a spectrophotometer.

N-Acetylsialic acid, *N*-glycolylsialic acid and *N*,*O*-diacetylsialic acid were used as reference substances. The molar absorbency indices were the same for *N*-acetylsialic acid and *N*,*O*-diacetylsialic acid in all four methods, while they were 10–20% higher for *N*-glycolylsialic acid. The most sensitive method was the resorcinol reaction, which allowed determinations of 1 μg. sialic acid with an accuracy of ±5%, while the direct Ehrlich reaction was the least sensitive method.

The main source of error was other carbohydrates, which gave additive absorbencies. Their influence was only negligible in the direct Ehrlich reaction, but this method was unsuitable in other respects. In the other three methods the influence of the carbohydrates was eliminated by reading the sample at two wavelengths and/or estimation of the amount of carbohydrates with an orcinol-sulphuric acid method (Svennerholm, 1956*b*). Keto-sugars gave with all the three methods the highest absorbencies but they occur only in negligible amounts in animal materials.

Bial's method and the resorcinol method are to be preferred for the determination of sialic acid, when it occurs together with hexoses, while the diphenylamine reaction is more convenient, when sialic acid is determined in samples also containing pentoses.

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Formation of Bile Acids from some Dihydroxy Steroids. By S. Lindstedt (introduced by the Committee). (*Department of Physiological Chemistry, University of Lund, Lund, Sweden*)

Tritium-labelled 7α-hydroxycholesterol has been prepared from labelled cholesterol according to Henbest & Jones (1948). It was injected in an aqueous suspension into rats with a bile-fistula. The bile acids were separated by reversed-phase chromatography (Sjövall, 1953; Norman, 1953). Cholic acid and chenodeoxycholic acid were identified as the main metabolites. The result is similar to that found when 3α:7α-dihydroxycoprostane was administered (Bergström & Lindstedt, 1956).

Bergström & Sjövall (1954) have found that cholic acid is not formed from chenodeoxycholic acid in the rat. When tritium-labelled 3α:7α-dihydroxycoprostanic acid was administered no label could be found in the cholic acid (S. Bergström, L. Krabisch & S. Lindstedt, to be published).

The results obtained support the theory put forward by Bergström (1955) that in the conversion of cholesterol into bile acids reactions in the ring system precede the total degradation of the side chain.

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Stability of Deoxyribonucleic Acid of Non-growing Rat Livers as Revealed by [^{14}C]Formate and [2-^{14}C]Glycine. By A. Sibatani.* (*Biochemistry Department, University of Glasgow*)

According to current views (Brown & Roll, 1955), non-growing rat liver tissue shows a very high RNA:DNA ratio for incorporation *in vivo* of labelled adenine, orthophosphate or orotic acid, while labelled formate and glycine yield much lower ratios. In slices of adult rat liver *in vitro* Mannell & Rossiter (1955) reported active incorporation of formate into both RNA and DNA.

* British Council Scholar, 1955–56.

If these findings reflect a metabolic instability of DNA in non-growing liver, they are inconsistent with the data of Kihara & Sibatani (1955) and Fresco, Bendich & Russell (1955), which suggest that both types of precursors are not released once they are incorporated into rat liver DNA.

In the present study an effective method of deproteinization of nucleic acids was employed involving precipitation of nucleoproteins at pH 4·5, extraction of nucleic acids with dodecylsulphate at

100° and pH 7, and shaking with chloroform in M-NaCl. After separation of RNA and DNA by alkaline digestion and subsequent acidification, followed by digestion with $HClO_4$, the bases were separated and freed from traces of amino acids by two-dimensional paper chromatography using butanol–ammonia–water (86:5:9) and simple distilled water as first and second solvents. In the regenerating livers of adult rats (150–200 g.) purines and thymine of DNA were extensively labelled both *in vivo* and *in vitro* by ^{14}C-formate and [2-^{14}C]glycine, while those of normal liver consistently gave very low specific activities. *In vivo* the RNA of normal liver showed little incorporation of isotope in 4 hr. and incorporation of the precursors into acid-soluble purines was also poor. In 24 hr. experiments *in vivo* and in all *in vitro* experiments with slices there was good incorporation of ^{14}C into RNA purines, the RNA:DNA ratio for adenine being 19–64 in normal and 1·7–6·2 in regenerating livers. Corresponding ratios for guanine in normal livers were usually lower than those for adenine, owing to less extensive labelling of RNA guanine.

Thus, even with glycine and formate, incorporation into DNA in normal liver tissue is definitely poor. These observations suggest a considerable metabolic stability of DNA in the absence of mitosis.

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The Effect of Dietary Fat on Various Signs of Vitamin E Deficiency in the Rat. By T. Moore, I. M. Sharman and R. J. Ward. (*Dunn Nutritional Laboratory, University of Cambridge, and Medical Research Council*)

Dam (1952) has stressed the importance of vitamin E as an *in vivo* antioxidant for fats, and the idea has gained ground that the effects of vitamin E deficiency cannot be fully developed unless the diet contains easily oxidizable fats. We have tested the general applicability of this theory in groups of rats which have been reared for 5 months upon either our usual vitamin E-deficient diet containing 10% of lard (Moore, Sharman & Ward, 1953) or upon the same diet modified by omitting lard, by increasing the lard to 30%, or by replacing lard by 10 or 30% of cod-liver oil. The various signs of vitamin E deficiency, which were consistent in each group, differed remarkably in the extent to which they were affected by the dietary fat. Haemolysis *in vitro* by dialuric acid, slight brown discoloration of the uterus and degeneration of the testes were observed even when the absence of essential fatty acids from the diet was indicated by scaly tails. Removal of fat from the diet, however, gave protection against dental depigmentation, as found by Granados & Dam (1945), and also against the degeneration of the kidneys first observed by Martin & Moore (1939), and now known to be made manifest by *post mortem* autolysis (Emmel, 1955). Cod-liver oil was distinguished as the only fat which caused the brown discoloration of the body fat (Dam & Granados, 1945). This oil was much more potent than lard in causing kidney degeneration, but was decidedly beneficial to the sex organs. Thus in the group receiving cod-liver oil at the 10% level both the uterus and testes were virtually normal. Presumably the cod-liver oil provided significant amounts of vitamin E (Brown, 1953) and influenced the various signs of deficiency according to the outcome in different tissues of the antagonism between the vitamin E and polyethylenic fatty acids which it simultaneously provided. No abnormalities were observed with the diet containing 10% of lard when weekly supplements of 2 mg. of DL-α-tocopheryl acetate were given.

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Indolylacetyl-Glutamine and other Indole Metabolites in Hartnup Disease. By J. B. JEPSON.
(*Courtauld Institute of Biochemistry, Middlesex Hospital, London, W.* 1)

A further genetically determined disease, Hartnup disease (also called Hart syndrome and H disease), has recently been added to the list of those dependent on paper-chromatographic demonstration of a characteristic urinary excretion pattern (Baron, Dent, Harris, Hart & Jepson, 1956; and references cited therein). Clinically this disease is characterized by pellagra-like skin rashes accompanied by intermittent cerebellar ataxia and mental deterioration. There is a constant renal aminoaciduria, quantitatively investigated by Evered (1956), who, however, erroneously reported the tryptophan excretion as zero; the typical amino acid excretion pattern constitutes the diagnostic test for Hartnup disease.

The amino acid excretion is accompanied in all cases by an equally typical excretion of indole derivatives. The urinary indole pattern as studied by paper chromatography (Jepson, 1955) shows four major Ehrlich-reacting components: indoxyl sulphate, 100–300 mg./day (normal, 50; see Rodnight & McIlwain, 1955); tryptophan, 100 mg./day (normal, 20); 3-indolylacetic acid (IAA), 50–200 mg./day—checked by plant growth activity (normal, 5); and *N*-(3-indolylacetyl)glutamine (IAc–Glu.NH_2), 30–150 mg./day.

IAc–Glu.NH_2 has not previously been reported as a urinary component. The structure of this material from Hartnup urine was deduced from the products of hydrolysis and checked by synthesis using the general method of Wieland & Hörlein (1955). Its excretion appears regulated by the IAA level, and it may represent the major pathway for the 'detoxication' of IAA, analogous to that of phenylacetic acid. Hartnup urine has no detectable content of indolylacetyl-glycine or indolyl-lactic acid. The indolic excretion was increased by tryptophan feeding. After a 4-day treatment with chlortetracycline only the indoxyl sulphate disappeared, to reappear in a few days.

In view of the known connexions between tryptophan, nicotinamide and pellagra it seems that the pellagra-like Hartnup disease represents a diversion of tryptophan away from its conversion to nicotinamide. (In the one case of true dietary pellagra which has been available for study the indole and amino acid excretion patterns were normal.) The primary defect may be in amino acid transport, or the gut flora, or the details of tryptophan dissimilation. Tryptophan 'tolerance' tests should decide between these possibilities and serve as a test for heterozygous carriers of Hartnup disease.

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Effect of Certain Hormonal Treatments on Glucose 6-Phosphatase Activity. By A. E. HARPER.*
(*Department of Biochemistry, University of Cambridge*)

The effects on glucose 6-phosphatase activity of several hormonal and dietary treatments have been studied in normal, diabetic and hypophysectomized rats. Glucose 6-phosphatase activity was estimated in homogenates of liver, and, in some cases, of kidney, using essentially the method of de Duve, Berthet, Hers & Dupret (1949).

In agreement with the results of other investigators (Ashmore, Hastings, Nesbett & Renold, 1956; Weber, Allard, de Lamirande & Cantero, 1956) liver glucose 6-phosphatase activity was found to increase during starvation (from about 4·5 to about 8 mg. of P released/g. of fresh liver/10 min.) and substantial rises in both total activity and activity per g. of fresh liver were observed in alloxan-diabetic rats. Changes, similar to those observed in alloxan-diabetic rats, could be produced by feeding rats, previously starved for 24 hr., solely on protein. Thus the increased glucose 6-phosphatase activity in alloxan-diabetic rats probably represents an adaptation to a diminution in the availability of glucose rather than a direct response to a lack of insulin.

A fall in the liver glucose 6-phosphatase activity (from about 4·5 to about 2·5 mg. P/g. fresh liver/hr.) was evident after hypophysectomy and similar low values were obtained in alloxan-diabetic rats that had been hypophysectomized. When rats were fed *ad lib.* no response to growth hormone injections could be detected. However, injections of hydrocortisone (1 mg./day) caused a definite increase in activity but the glucose 6-phosphatase activity of the liver was not restored to normal by this treatment. Subsequently injections of thyroxine were found to give a response similar to that obtained with hydrocortisone. Combined injections of hydrocortisone and thyroxine increased the liver glucose 6-phosphatase activity of hypo-

* Fellow of the Rockefeller Foundation and the National Research Council, Washington, D.C. Permanent address: Department of Biochemistry, University of Wisconsin, Madison.

physectomized rats to a level above that of the intact normal rat.

These results show that glucose 6-phosphatase activity in the liver is influenced by endocrine factors which include hydrocortisone and thyroxine acting directly or indirectly. No evidence was obtained that growth hormone was one of the factors involved.

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Glutamic Dehydrogenase in the Developing Chick Embryo. By J. B. SOLOMON. (*Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, London, S.W.* 3)

The ontogenic sequence of carbohydrate and protein as primary energy sources during the development of the chick embryo (Needham, 1931) may be accompanied by the appearance of enzymes concerned with the conversion of carbohydrate to protein. The yolk of the hen's egg has been found to contain glutamic dehydrogenase (GDH) which catalyses the conversion of α-ketoglutaric acid to glutamic acid in the presence of ammonia and reduced diphosphopyridine nucleotide (DPNH). The transfer of this enzyme through the yolk sac to the embryo has been followed during the development of the chick embryo. Homogenates of blastoderms, embryos and extra-embryonic tissues were assayed for GDH activity by measuring the reduction in optical density at 340 mμ. due to the oxidation of DPNH with α-ketoglutaric acid as the substrate (Olson & Anfinsen, 1952; Hogeboom & Schneider, 1953).

Some GDH activity was present in blastoderms at the primitive streak stage and increased rapidly in the yolk sac until the fourth day of incubation. Traces of GDH activity were found in ten-somite embryos, but the activity per embryo did not increase significantly until the fifth day of incubation. After the ninth day the embryo possessed more GDH activity than its extra-embryonic tissues.

In the blastoderm and extra-embryonic tissue the GDH activity per unit wet weight and per mg. protein decreased markedly until the second day of incubation, and after rising to a peak on the fourth day fell to a constant level; the rise in activity between the second and fourth day is associated with the rapid development of the vascular area. In the embryo, GDH activity per unit wet weight and per mg. protein was maximal after 2 days' incubation, then fell rapidly until the fourth day, but showed a slight increase during the sixth to twelfth day of development. The intensity of absorption of GDH by the developing chick embryo shows some resemblance to that of total carbohydrate (Needham, 1931).

GDH activity is present in the liver, heart and brain of 10-day chick embryos and in each organ the activity per mg. protein increases during the period from 10 days' incubation to 3 days after hatching.

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The Absorption of 7-Dehydrocholesterol in the Rat. By J. GLOVER and D. STAINER. (*Biochemistry Department, University of Liverpool*)

The absorption of sterols across the intestinal mucosae of the guinea pig has previously been shown to involve an exchange and transfer of the sterol between the lipoproteins of the cell membrane, organelles and groundplasm (Glover & Green, 1954, 1955). A similar study has now been carried out on the rat. Since the rat intestine contains only a trace of 7-dehydrocholesterol ($\Delta^{5,7}$-cholestadiene-3-β-ol) (Glover, Glover & Morton, 1952), the absorption of this sterol across the mucosae was readily followed spectrophotometrically.

Groups of two male rats were fasted 15 hr. and each animal was given orally 200 mg. 7-dehydrocholesterol in 1·0 ml. arachis oil. Five hours after dosing, the animals were killed, the top halves of the small intestines were removed, flushed out with 0·25 M sucrose solution and slit longitudinally. The mucosae were scraped off in the cold (0–5°), comminuted in a Potter-Elvehjem homogenizer and fractionated in 0·25 M sucrose solution by the Schneider–Hogeboom (1950) technique to yield fractions: cell debris + nuclei, mitochondria, microsomes, groundplasm and a supernatant fatty layer.

The lipids were extracted from each fraction and examined for 7-dehydrocholesterol (Glover *et al.* 1952), cholesterol and total Δ^7-sterols [Δ^7-cholestenol (lathosterol) and $\Delta^{5,7}$-cholestadienol] (Idler & Baumann, 1952) and total sterols (Kenny, 1952). The ratios of total sterols to 7-dehydrocholesterol (R_1) and to the total Δ^7-sterols (R_2) were determined for each cell fraction.

The values for R_1 in the various fractions from the controls which yielded no fatty layer and contain only small amounts of 7-dehydrocholesterol were: 21–38; 25–26; 20–59; 37–56; 33–41; and for R_2: 7–12; 7–9; 7–8; 6–12; 11–14 respectively. In three experiments with dosed animals, the corresponding ratios were much lower; for R_1 the values were: 3·5 to 7·4, 3·5 to 4·6, 2·4 to 3·8, 6·3 to 6·5 and 5·2 to 11·7 (fatty layer) respectively; and for R_2: 1·3 to 2·1, 1·3 to 3·8, 1·1 to 1·7, 3·2 to 5·6 and 4·0 to 5·0 (fatty layer) respectively. The results show clearly (*a*) that the administered sterol has become associated with each cellular fraction, and (*b*) that an exchange of the above three sterols between the organelles and groundplasm has occurred, since the values for R_1 in the various fractions and particularly also for R_2 are quite uniform. This confirms the previous work with guinea pigs (Glover & Green, 1954, 1955) and indicates that the absorption of sterols in this species also takes place at the molecular level, by an exchange and transfer on lipoproteins.

Evidence was also obtained for the reduction of some 7-dehydrocholesterol to lathosterol.

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The Purification of Myokinase. By O. H. Callaghan (introduced by G. Weber). (*Department of Biochemistry, University of Sheffield*)

Previous authors (Colowick & Kalckar, 1943; Kalckar, 1943; Noda & Kuby, 1955) have reported the purification of myokinase. The following method gives a purer preparation, in which ultracentrifugation and zone electrophoresis (Flodin & Porath, 1954) revealed a single component.

All operations are carried out in the cold. Fresh chilled rabbit muscle is minced and extracted with 1·5 vol. of 0·05M-KCl. The extract is dialysed and fractionated with *n*-propanol according to Askonas (1951). At pH 6·5 and $-5°$, the fraction precipitated in 40 vol. % *n*-propanol is centrifuged down at $-5°$, and suspended in 0·033M phosphate buffer pH 7·4, and dialysed against the same buffer for 3 hr. A flocculent precipitate appears, which contains the enzyme. This is collected and the inactive supernatant discarded. The precipitate is suspended in distilled water (0·25 vol. per vol. of original extract), dissolved at pH 2 by adding 2N-HCl, treated with 0·04 vol. of 5M-NaCl and readjusted to pH 6·5 with 2N-NaOH, when a precipitate reappears. The clear supernatant is a stable enzyme solution. The precipitate is re-extracted as above for further enzyme. The combined solutions are fractionated in a calcium phosphate gel column (Swingle & Tiselius, 1951) using Whatman's cellulose powder as inert packing material instead of the conventional Hyflo Supercel. A column 15×6 cm.^{2} is adequate for the protein derived from 500 g. muscle. After washing the protein on the column with 0·2M-NaCl, a single peak (absorbing light at 280 mμ.) is eluted with 0·025M phosphate buffer pH 7·0 in 0·2M-NaCl, the fractions being collected in polythene tubes. The protein is further fractionated with $(NH_4)_2SO_4$, the fraction between 0·65 and 0·85 saturation being retained.

The procedure yields 40 % of the enzyme present in the extract with 60-fold purification.

Paper electrophoresis indicates that the protein is isoelectric at pH 4·3. An uncorrected sedimentation constant $s = 1{\cdot}7 \times 10^{-13}$ was obtained.

Glass and cellophane denature the pure enzyme in solution. Solutions may be safely stored in polythene tubes, and dialysed in collodion tubes (previously coated with haemoglobin or cytochrome *c*). At pH 7·5 and at 30°, the enzyme dismutes 220 000 moles ADP/100 000 g. protein/minute.

Adenosine triphosphatase, 5′-adenylic deaminase, and creatine phosphokinase could not be detected in the preparation.

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The Biological Activity of Insulin Peptides. By D. S. H. W. NICOL* and L. F. SMITH. (*Department of Biochemistry, University of Cambridge*)

Lens (1949) showed that alanine was liberated from insulin by the enzyme carboxypeptidase. He found the residual molecule to retain only 15% biological activity. Harris & Li (1952) found that under conditions in which alanine was the main product, activity was retained. In addition, asparagine was slowly liberated accompanied by a progressive decrease in activity. We have repeated this and confirmed that insulin without the *C*-terminal alanine residue (DA-insulin) retains activity. Carboxypeptidase acts more readily on acetyl-insulin removing both alanine and asparagine. Acetyl-insulin is active (Fraenkel-Conrat & Fraenkel-Conrat, 1950) and this biological activity was confirmed. Removal of the *C*-terminal amino acids caused a loss of the biological activity.

The action of trypsin on insulin results in the loss of alanine and a heptapeptide Gly.Phe.Phe.Tyr.Thr.Pro.Lys from the phenylalanyl chain of the molecule (Sanger & Tuppy, 1951; Harris & Li, 1952). Using this method we have obtained a yield of 15% of the heptapeptide, as estimated by the tyrosine content. The action of trypsin on esterified insulin gave an increased yield of the heptapeptide. The removal of zinc from insulin renders it more susceptible to the action of leucine amino-peptidase (private communication from Dr E. L. Smith). Insulin was treated with versene (Sluyterman, 1955) to remove the zinc. Trypsin acted on the zinc-free insulin, yielding about 40% of the heptapeptide. The residue after precipitation at pH 5·4 was fractionated by reverse phase chromatography using a column of silane-treated Celite 545 with the system of *sec.*-butanol-0·05M trichloroacetic acid (2:5) (Andersen, 1954). A large peptide moving faster than insulin was isolated. This proved to be the insulin molecule without the terminal heptapeptide and alanine molecules (DHA-insulin). DHA-insulin and the heptapeptide were tested in dosages up to 2 mg. (original insulin 22 units/mg.) but showed very little or no hypoglycaemic effect on rabbits or any antagonistic or synergistic effect when combined with insulin. Controls of zinc-free insulin as such, and after passing through a similar column showed biological activity.

DA-insulin and DHA-insulin formed fibrils, like ordinary insulin (Waugh, 1950), which were similarly inactive.

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***cis*-Isomers of Vitamin A in some Marine Crustacea.** By P. A. PLACK, L. R. FISHER, KATHLEEN M. HENRY and S. K. KON. (*Unit for Biochemical Research bearing on Fisheries Problems* at the National Institute for Research in Dairying, University of Reading*)

The *cis-trans* isomers of vitamin A may be divided into two groups by their reaction with maleic anhydride (Robeson, Cawley *et al.*, 1955): the 'fast' reacting group, all-*trans*, 9-*cis*- and possibly 7-*cis*- (Karrer numbering), and the 'slow' reacting group, 11-*cis*-, 13-*cis*-, 9:13-di-*cis*- and 11:13-di-*cis*-vitamin A. Plack (1956) has described a method of determining the percentages of 'fast' and 'slow' reacting components in a mixture.

Vitamin A from the Crustacea *Meganyctiphanes norvegica, Thysanoessa raschii, Pandalus bonnieri, P. borealis* and *P. montagui* contained 70–95% of 'slow' components, whereas vitamin A from fish and whale livers contained 10–40%. The crustacean vitamin A was further differentiated by values of 3·5–4·2 for the ratio *E* (Carr-Price reaction at 618 mμ.)/E (u.v. maximum in *n*-hexane), and by broad absorption maxima in the range 315–325 mμ. Isomerization with iodine in *n*-hexane for 1 min. resulted in a shift in $\lambda_{max.}$ to 325 mμ., and in a decrease of the ratio to 2·9–3·0 through an increase in extinction at $\lambda_{max.}$ u.v. of up to 1·5 times. These properties are consistent with vitamin A being largely in the form of 'hindered'

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cis isomers (Oroshnik & Mebane, 1954). *M. norvegica* and *T. raschii* contain only small amounts of 11:13-di-*cis* (Oroshnik, 1956; cf. Fisher, Henry, Kon, Plack & Thompson, 1955) and the opsin test (Hubbard & Wald, 1952–53) shows that the bulk of the vitamin A from these and from the *Pandalus* species is neovitamin A*b* (11-*cis* according to Oroshnik, 1956).

Vitamin A from fish and whale livers gave $E_{C.P.}/E_{u.v.}$ ratios of 2·3–2·9 with no change on isomerization.

Biological assays by the rat-growth method confirmed the presence of *cis*-isomers. For equal Carr-Price values, the biological potencies of the vitamin A from *M. norvegica*, *T. raschii*, *P. bonnieri*, the 'slow' components from *M. norvegica* and *T. raschii* were, respectively, 32, 35, 44, 25 and 29% of that of all-*trans* vitamin A (cf. Ames, Swanson & Harris, 1955).

Wald & Burg (1955) have recently found neovitamin A*b* in lobster eyes, and Lambertsen & Braekkan (1955) have reported an isomer in *P. borealis*.

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Changes with Age in the Protein Composition of Rat Brain. By Doris H. Clouet and M. K. Gaitonde. (*Neuropsychiatric Research Centre, Whitchurch Hospital, Cardiff*)

A study was made of changes in the protein content and composition of rat brain in a series of rats of different ages from foetus to adult. The nitrogen content in relation to wet weight, dry weight, and lipid-free weight of the brain was measured in the whole brain and in six areas of the brain representing different stages of ontogenetic development: cortex, medulla, cerebellum, olfactory bulb, mid-brain and hypothalamus. As an index of the chemical composition of the protein, two amino acids, arginine and histidine, were determined in protein hydrolysates from the same brains. Arginine was estimated in the hydrolysate directly; histidine required a preliminary adsorption on charcoal to eliminate contaminating substances.

The nitrogen, and consequently the protein content, of rat brain was related to the age of the animal. The absolute amount of nitrogen showed a regular increase with age. When the protein content was related to the total solids, a peak was found at the seventh day after birth.

The protein content of each area of brain was also related to the age of the animal. The protein content of the brain of young rats decreased in the order: medulla > hypothalamus > cerebellum > mid-brain > olfactory bulb > cortex. In older rats the order was: mid-brain > cortex > cerebellum > hypothalamus > olfactory bulb > medulla. These results support the view that the phylogenetically older parts of the brain develop before the newer parts as the brain matures.

The protein composition also varied with the age of the animal. The histidine content of the brain proteins was lower in the first day after birth than in the foetus; it then increased to regain the foetal level in 20 days, and rose to a constant level about one-third higher at 80 days. The arginine content of the brain proteins showed a similar pattern of change. On separating the rats into two groups at a dividing age of 40 days, a significant increase was observed in the proportion of arginine and histidine in the brain proteins of the older animals. A similar increase was observed in the proteins of different areas of the brain of the older rats.

The Cytochrome System in the Plasma Membrane of *Staphylococcus aureus*. By P. MITCHELL and JENNIFER MOYLE. (*Zoology Department, University of Edinburgh*)

Controlled autolysis of *Staphylococcus aureus* in a medium of high osmotic pressure, followed by dilution with water, causes the cell walls to break into two hemispherical parts (Mitchell & Moyle, 1956). The inner protoplasts burst, but the protoplast (plasma-) membranes do not disintegrate and may be collected by centrifugation. On washing with distilled water the plasma-membranes break down to small particles, indistinguishable from the 'small particle' lipo-protein fraction which was isolated from mechanically disintegrated staphylococci (Mitchell & Moyle, 1951) and which we previously suggested might be the plasma-membrane material. The dry weights and morphology of cell wall and 'plasma-membrane' material produced from 100 mg. cells by mechanical disintegration (M) or by autolysis (A) were: cell wall: M, 16·6 mg. spherical shells; A, 12·5 mg. hemispherical shells. Plasma-membrane: M, 9·6 mg. small particles; A, 10·2 mg. spherical shells. Since the cells are *ca.* 0·7 μ. in diameter, the dry thickness of the plasma-membrane is *ca.* 50 Å and its composition shows that it corresponds to abcut a monolayer of lipid and a monolayer of protein.

The extinction of the reduced cytochrome system at 425 mμ. shows that at least 90 % is located in the plasma-membrane fraction, confirming our previous observations on the 'small particle' fraction (Mitchell, 1954). The ratios of a number of enzyme activities measured in the plasma-membrane or 'small particle' fraction to that measured in the remaining fractions are as follows: succinic dehydrogenase, >9; lactic dehydrogenase, 8–9·5; 'malic' enzyme, >9; malic dehydrogenase, >9; formic dehydrogenase, >9; acid phosphatase, >9; α-glycerophosphate dehydrogenase, 5–7; glucose-6-phosphate dehydrogenase, 0·03; glucose-6-phosphatase, 0·1.

It follows that part of the protein of the plasma-membrane is constituted of enzymes, the active centres of which are probably at least as close as 50 Å to the outer medium, and may be able to make contact with it. Since the end-products of metabolism of staphylococci are lactic, formic and succinic acids, we suggest that the corresponding dehydrogenases, which are concentrated in the membrane, may be concerned with the movement of their substrates through the membrane. The occurrence of the cytochrome system in the membrane supports hypotheses of the implication of the cytochrome system in ion transport.

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The Oxidation of β-Carotene by a Lipoxidase-Linoleate System. By J. FRIEND. (*Low Temperature Station for Research in Biochemistry and Biophysics, University of Cambridge, and Department of Scientific and Industrial Research*)

The bleaching of carotene by lipoxidase in the presence of unsaturated fat has been known for some time and has found extensive application as an assay method for lipoxidase (literature reviewed in Holman & Bergstrom, 1951). Lipoxidase has also been suggested as the enzyme causing carotene destruction in alfalfa (Mitchell & Hauge, 1946).

We have partially bleached chromatographically pure β-carotene in the presence of sodium linoleate and purified soy-bean lipoxidase using a system based on the assay method of Blain, Hawthorn & Todd (1953), and extracted, with ether, the yellow products from the saponified reaction mixture. Chromatography of this ether extract has shown the presence of several epoxides, some of which have very similar absorption maxima in CS_2 to that of Aurochrome, the di-furanoid epoxide of β-carotene (Karrer & Jucker, 1945), and are possibly *cis- trans* isomers of this pigment.

The three major pigments have spectra similar in shape to β-apocarotenals obtained by partial degradation of β-carotene with hydrogen peroxide and osmium tetroxide (Redfearn, 1954; Friend, 1955). They show a bathochromic displacement on transference from petrol to alcohol and are reduced by metal hydrides, properties consistent with compounds containing a carbonyl group conjugated with a conjugated polyene chromophore. However, their rates of reduction with metal hydrides correspond with those of unsaturated

ketones rather than unsaturated aldehydes (Friend & Swain, unpublished) and they do not form Schiff's bases with methylamine; this formation is characteristic of unsaturated aldehydes (Pitt, Collins, Morton & Stok, 1955).

If our compounds are ketonic then it is possible that they have a *retro* structure (Oroshnik, Karmas & Mebane, 1952). Experiments designed to test this are in progress.

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Anserinase and other Dipeptidase Activity in Skeletal Muscle of Codling *(Gadus callarias)*.

By N. R. Jones. (*Torry Research Station, Aberdeen*)

Anserinase in the muscle of codling (Jones, 1954, 1955) differs from the well-known, metal-activated dipeptidases of swine intestinal mucosa. The enzyme is absent from that tissue, the muscle of rat and rabbit and also from muscles of some other fish species examined, including lemon sole (*Pleuronectes microcephalus*) which has a free amino acid composition similar to codling.

Observations on anserinase activity in codling muscle have now been extended using an improved method of assay. The sensitivity of dipeptidase measurement, as described in the Fleisher (1953) modification of the procedure of Schwartz & Engel (1950), was considerably increased by a simple alteration of reaction conditions. Deproteinized incubate was allowed to react with Moore & Stein (1948) ninhydrin reagent for 120 min. at 35° instead of 20 min. at 100°. Anserine gave a negligible absorption at 570 mμ. at 35°, whereas it increased throughout the reaction-time at 100°. Improvement was not obtained with other dipeptides tested.

Aqueous extracts of codling muscle (Jones, 1955), preincubated with $10^{-3\cdot8}$ M-Zn^{2+}, have been shown to contain a hitherto unobserved, low anserine-cleaving activity-peak at pH 3·2 in addition to the anserinase peak at pH 7·3–7·5. Such preparations had dipeptidase activity also towards glycylleucine, leucyltyrosine, alanylglycine, leucylglycine and carnosine with pH optima in similar regions. With the exception of carnosine, which was hydrolysed in a pattern similar to anserine, activity towards the dipeptides at pH 3–4 approached that at pH 7–8, the exact positions of the peaks varying slightly. Histidylhistidine was hydrolysed in neither pH range.

Dipeptidase activity in the pH 3–4 region could be removed by isoelectric precipitation or differential heating. Activity in the pH 7–8 range remained in solution and, in subsequent purification stages, hydrolysis of peptides other than anserine has closely paralleled anserinase activity. Anserine cleavage by a Zn^{2+}-activated, acetone-fractionated system, as estimated chromatographically, was competitively inhibited by glycylleucine. It thus appears that anserinase, while differing from other known metal-activated dipeptidases, has a low specificity.

The work described in this paper was carried out as part of the programme of the Food Investigation Organization, Department of Scientific and Industrial Research.

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Nitrogen Metabolism in Jack Bean (*Canavalia ensiformis*). By J. H. JOHNSTONE. (*Department of Biochemistry, Queen's College, Dundee, University of St Andrews*)

Jack beans germinated under standard conditions were harvested at regular intervals of 2 days for a period of 2 weeks. The changes in protein and non-protein nitrogen in the cotyledon and in the embryo were studied.

The tissue was dried *in vacuo*, ground and extracted with aqueous alcohol (80%, v/v). The extracts were freed from alcohol by shaking with chloroform (3 vol.) and the non-protein nitrogen in the aqueous layer studied by semi-quantitative two-dimensional paper chromatography, using the solvent system of Levy & Clung (1953). A quantitative analysis of the hydrolysed non-protein fractions was carried out using ion-exchange resin Dowex 50 × 8 (Moore & Stein, 1951) and later Zeo-Karb 225 (Campbell, Jacobs, Work & Kressman, 1955).

Paper partition chromatography revealed the usual amino acids and amines present in plant tissue (see Stewart, Zacharius & Pollard, 1955). Canavanine is the predominating amino acid and large amounts of homoserine and glutamic acid are also present in the non-protein fraction of the cotyledon. There are a number of unidentified ninhydrin-positive compounds.

The main differences in distribution of amino acids between the ungerminated embryo and the cotyledon are as follows: canavanine is absent from the embryonic tissue, while homoserine is the most abundant amino acid. The embryo contains a high concentration of a compound which on hydrolysis yields homoserine.

This compound resembles the unknown 'O' of Virtanen & Linko (1955), which on hydrolysis yields homoserine. Chromatography of the hydrolysates of the extracts of both cotyledon and embryo show a considerable increase in the concentration of homoserine. The embryo non-protein extract contains little basic ninhydrin-positive material.

As germination proceeded there was a decrease in the concentration of canavanine in the cotyledon and an increase in the ninhydrin-positive components of the hydrolysed extracts.

The total globulin of the cotyledon, prepared by the method of Sumner (1919), was analysed for amino acid composition by paper partition chromatography and by ion exchange resin Zeo-Karb 225. Canavanine and homoserine were absent.

It is suggested that canavanine is formed during seed formation and that it functions as a storage component of nitrogen and carbon for the developing embryo and may be broken down via homoserine. Incubation of canavanine with an aqueous extract of jack bean cotyledon yielded mainly homoserine and a trace of alanine.

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The Appearance of H Specificity following the Enzymic Inactivation of Blood Group B Substance. By WINIFRED M. WATKINS. (*Lister Institute, London, S.W.* 1)

Iseki & Masaki (1953) using an A-decomposing enzyme from *Clostridium tertium* reported that when this enzyme was allowed to act on A substance, the A specificity disappeared and O (H) activity was developed. It has now been shown that when purified human B substance (Gibbons & Morgan, 1954) is treated with the B-decomposing enzyme from *Trichomonas foetus* (Watkins, 1953) the disappearance of B activity is accompanied by a concomitant development of H specificity.

The B-decomposing enzyme was separated from the H enzyme, which is also present in crude extracts of *T. foetus*, by zone electrophoresis at pH 7 on a starch column. The H specific activity which appeared after treatment of B substance with the enzyme was detected by means of agglutination inhibition tests using immune rabbit or natural eel anti-H reagents or by the development of precipitation with rabbit anti-H serum. Inhibition tests indicated that the H activity developed was equivalent to about 10% of the activity of a purified H substance. Further treatment with an H enzyme preparation resulted in the disappearance of the newly formed H properties. The action of the B enzyme on B substance can be inhibited by galactose (Watkins

& Morgan, 1955), and when this sugar was present in the reaction mixture there was no development of H specific activity. Treatment of B substance with the B enzyme did not lead to the development of A activity as measured by either iso-agglutination or haemolysis inhibition tests, but the treated material had acquired a slight capacity to cross-react with horse anti-pneumococcus Type XIV serum; the degree of precipitation with this reagent was greatly increased after subsequent exposure to the H enzyme.

Reducing sugars equivalent to 5% glucose were liberated from the B substance by the action of the B enzyme. On dialysis through a cellophane membrane the H activity remained in the indiffusible portion. Chromatographic examination of the diffusible material showed that galactose was the sugar liberated in greatest amount by the B enzyme but small quantities of fucose and *N*-acetylhexosamine could also be detected. Subsequent treatment of the indiffusible, H active, material with H enzyme resulted in the liberation of fucose and a small quantity of *N*-acetylhexosamine (cf. Watkins, 1955).

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A New Amino Sugar present in the Specific Polysaccharides of some Strains of *Chromobacterium violaceum*. By M. J. CRUMPTON and D. A. L. DAVIES. (*Microbiological Research Establishment, Porton, Wiltshire*)

In the specific lipopolysaccharides of some strains of *Chromobacterium violaceum*, a substance reacting as a 2-amino sugar (Elson & Morgan, 1933) has been detected which is not glucosamine, galactosamine, nor the amino sugar described by Strange & Dark (1956). The purified lipopolysaccharide of strain N.C.T.C. 7917 contained 16% total amino sugar estimated as glucosamine (Rondle & Morgan, 1955*a*). Lipopolysaccharide hydrolysates (0·5N-HCl, 16 hr., 100°) were examined by paper chromatography in several solvent systems; when sprayed to reveal reducing sugars, glucosamine, galactose and glucose were found, and an aldoheptose sugar not yet identified. When sprayed with hexosamine reagents (Partridge, 1948) two spots were found, one due to glucosamine and another differing in position from glucosamine and galactosamine in all solvent systems used. The $R_{glucose}$ values for this component in butanol–pyridine–water (6:4:3) and in phenol-NH_3 were 1·03 and 2·14 respectively. The new amino sugar was stable to 6N-HCl hydrolysis for 16 hr. at 100°.

Hydrolysates were also analysed on resin columns (Zeo-Karb 225) by a modification (Rondle & Morgan, 1955*b*) of the method of Gardell (1953). After the neutral sugars had been eluted, columns were developed with 0·33N-HCl which separated glucosamine from a slower running component. When galactosamine was added to a hydrolysate, three components could be separated completely from one another, emerging in the order glucosamine, galactosamine and the new amino sugar. The ratio of the new component in the lipopolysaccharide to the glucosamine present was 6·4:1, and the absorption spectrum of the unidentified substance on reacting with alkaline acetyl-acetone and Ehrlich's reagent (Rondle & Morgan, 1955*a*) was identical with that given by glucosamine.

The amino sugar was present in the lipopolysaccharide in N-acetylated form, the maximum N-acetylhexosamine colour (Aminoff, Morgan & Watkins, 1952) being obtained (18%) after 12 hr. hydrolysis at 100° with N-acetic acid. The acetylated sugar could readily be separated on paper chromatograms from N-acetylglucosamine and N-acetylgalactosamine, and had a $R_{glucose}$ value of 1·66 in butanol–pyridine–water as solvent.

When the lipid and polysaccharide moieties were separated as described by Davies (1956) all of the glucosamine could be accounted for in the lipid fraction, the new sugar being the only amino sugar present in the specific degraded polysaccharide hapten.

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The Structure of an Amino Sugar present in Certain Spores and Bacterial Cell Walls. By R. E. STRANGE. (*Microbiological Research Establishment, Porton, Wiltshire*)

The substance (AS) was discovered in 6N-HCl hydrolysates of 'spore peptide' (Strange & Powell, 1954) and bacterial cell walls (Cummins & Harris, 1954, 1955). It has been isolated in crystalline form and its 'hexosamine value' determined (Strange & Dark, 1956). AS, crystallized (×3) from ethanol-H_2O (90%, v/v) and dried *in vacuo* over P_2O_5 at 60°/1 hr. contained C 40·5, H 7·06, N 5·28%, corresponding to $C_9H_{19}O_8N$; mol.wt., 269. Barger's method showed that the molecular weight was not a multiple of this. Purity was confirmed by paper chromatography with five solvent systems. Electrometric titration suggested a carboxyl and an amino group (approx. p*K*'s of 2·6 and 8·8, respectively). The amino group was shown to be free by the liberation of nearly quantitative yields of NH_3 on alkaline distillation (Tracey, 1952). The Elson & Morgan (1933) reaction was positive, indicating a vicinal carbonyl group. Determinations of reducing power and HCN addition (Militzer, 1946) gave results of 29 and 23%, as glucose, respectively. Quantitative acetylation showed 3·8 hydroxyl and amino groups. The isolated, acetylated AS gave a Morgan & Elson (1934) reaction. Oxidation of AS with periodate liberated 0·93 mol. formaldehyde (O'Dea & Gibbons, 1953), 1·7 mol. formic acid and ammonia. Oxidation with ninhydrin (Stoffyn & Jeanloz, 1954) gave a product which appeared to be a substituted pentose.

The results suggested hexosamine with a side chain having a free carboxyl group. The stability of AS in hot, moderately strong acids suggested a C–C or ether linkage. No volatile alkyl halide was released in the Zeisel method, but after treatment of AS with HI and red phosphorus, a fatty acid was extracted from the products which had the same R_F on paper chromatograms (Reid & Lederer, 1951) as propionic acid. When AS was heated in H_2SO_4 (86%, v/v) at 100° for 17 min. and *p*-hydroxydiphenyl + Cu^{2+} (Barker & Summerson, 1941) added to the cooled solution, a purple colour developed, characteristic of acetaldehyde. Compared with standards, this colour indicated the presence of approx. 25% combined lactic acid in AS.

It is probable that the side chain is not associated with carbon atoms 1, 2, 5 or 6. Kuhn, Gauge & Baer (1954) report that 4-*O*-derivatives of *N*-acetylglucosamine fail to give a Morgan–Elson reaction and if this finding is of general application to *N*-acetylhexosamines, then in AS, the group is associated with carbon 3. A possible structure for AS is 3-*O*-α-carboxyethylhexosamine, $C_9H_{17}O_7N$, H_2O. Confirmation by synthesis is required.

My thanks are given to Major L. H. Kent and Dr D. Herbert for helpful suggestions.

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The Effect of pH on the Hydrolysis of *n*-Hexanamide by an Amidase of Rabbit Liver. By K. WHITE. (*Physiology Department, Medical School, University of Birmingham*)

The kinetics of hydrolysis of *n*-hexanamide by an amidase of rabbit liver (Bray, White & Wood 1955) have been investigated in the range pH 5–9. The Michaelis constant, K_m, the equilibrium constant for inhibition by 'excess' substrate, K_{ss}, and the theoretical maximum velocity, V, were determined.

The value of K_m was found to be 80 mM in the region pH 5–6, decreasing to 5 mM at pH 8·5. A plot of $-\log K_m$ against pH showed that this variation could be accounted for by the presence of an ionizing group in the enzyme possessing a pK value of 7·7 in the free enzyme and 6·6 in the enzyme-substrate complex (cf. line *C*, fig. 2; Dixon, 1953).

V was found to be independent of pH, which indicates that the activation of the enzyme-substrate complex does not involve an ionizing

group in the enzyme with a pK value in the range 5–9, and that neither hydrogen nor hydroxyl ions are concerned in a rate determining step of the hydrolysis.

The value of K_{ss} was approximately 40 mM over the pH range investigated. The second molecule of substrate adsorbed by the enzyme does not, therefore, affect the pK value of an ionizing group of the enzyme. The fact that at pH 5–6 the value of K_{ss} is less than that of K_m cannot be accounted for by the theory of step reactions if the adsorption of each of the two molecules of substrate by the enzyme proceeds independently, and it is concluded that adsorption of the second molecule is co-operative.

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Effect of Oral or Parenteral Administration of a Massive Dose of Vitamins D on the Distribution in Rat Tissues. By E. M. Cruickshank and E. Kodicek. (*Dunn Nutritional Laboratory, University of Cambridge, and Medical Research Council*)

Weanling rats were given, by stomach tube or intramuscular injection, a single dose of 1 mg. vitamin D_2 or D_3, dispersed in Tween 80 and water. The pooled livers, intestines (minus contents), kidneys and faeces from groups of three rats, killed 24 and 48 hr. after dosing, were assayed for vitamin D by paper-chromatography (Kodicek & Ashby, 1954) or by bioassay on rats (Bourdillon, Bruce, Fischmann & Webster, 1931).

When vitamin D_2 was given perorally, the livers, intestinal tissue and kidneys contained, at 24 hr., 119, 4·4 and 3·7 μg., and after 48 hr., 67, 1·6 and 2·5 μg./rat, respectively. When the vitamin was injected, the corresponding figures were 98, 2·4 and 3·6 μg. after 24 hr., and 76, 2·5 and 4·0 μg. after 48 hr. The faecal excretion of vitamin D in the 'peroral' group was 155 and 99 μg./rat, at 24 and 48 hr., respectively; in the 'injected' group the corresponding values were 5·4 and 64 μg. A similar distribution was observed with vitamin D_3.

The liver vitamin in the 'peroral' group decreased as in previous experiments (Cruickshank, Kodicek & Armitage, 1953), by 40% between 24 and 48 hr. Isotopic experiments have indicated that this decrease was due to destruction of the vitamin in the liver (Kodicek, 1956). In the 'injected' group the decrease was less pronounced: it is possible that the maximum transfer of vitamin to the liver had not been detected, if this occurred within 24 hr. after injection. The appearance of vitamin D in the intestinal tissue after injection may indicate that its known influence on the mineral metabolism of intestinal cells is of a local nature. In this connexion, it may be of significance that little or no vitamin finds its way into the intestinal contents, at least during the first 24 hr. Its appearance in faeces at 48 hr. may be derived from excretion via the bile (Heymann, 1937). The kidneys of the 'injected' and 'peroral' groups contained significant amounts of vitamin D, and, since none appears in urine, the kidneys act as a barrier. The vitamin in the kidneys may serve a metabolic function associated with reabsorption of P by the kidney tubules, as Harrison & Harrison (1941) suggest.

The distribution of vitamin D in the tissues is not significantly different, whether the vitamin is administered in oil (Cruickshank, *et al.* 1953, 1955; Kodicek, 1954) or in aqueous dispersion.

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The Fate of ^{14}C-Labelled Vitamin D_2 in the Rat. By E. KODICEK. (*Dunn Nutritional Laboratory, University of Cambridge, and Medical Research Council*)

Balance experiments, in which vitamin D was determined by bioassay, have shown that only 20–30% of a dose can be accounted for as biologically active vitamin D (Cruickshank, Kodicek & Armitage, 1953). The preparation of uniformly ^{14}C-labelled ergocalciferol (Kodicek, 1955) made it possible to study in more detail the fate of the administered vitamin. A single dose of [^{14}C]-ergocalciferol, 1 mg. dissolved in arachis oil, was given *per os* to two rachitic rats, and 24 hr. later, the excreta and tissues, after fractionation, were analysed by paper chromatography (Kodicek & Ashby, 1954) and the usual isotope techniques. All the ^{14}C was recovered, but only 30% as vitamin D. In the body tissues and faeces, 10·6 and 19·7%, respectively, of the label was as vitamin D, and 9·2 and 59·6% as unidentified breakdown products. In the urine, no vitamin D was detected, but 2·1% of ^{14}C was present as breakdown products, equally distributed between the unsaponifiable matter and fatty acid fraction. The main bulk of the body vitamin was in the liver, 5·7%; bones, 1·4% (biological assay); intestines, 0·6%, and kidneys, 0·2% of the dose. Blood contained 1·2% and other tissues, 1·5% of the label, most likely as vitamin D.

The main portion of the breakdown products was in the faeces; on fractionation, 47% of the dose were soluble in 50% ethanol and 12·6% soluble in absolute ethanol. Since a similar pattern of breakdown products was found in the liver, it is likely that at least some of the radioactive substances originated in the liver and were excreted through the bile. The intestinal tissue contained 0·9% of the label as breakdown products, but, in contrast to liver, they occurred in the unsaponifiable matter obtained from ether extracts and were digitonin-precipitable. The turnover in the liver is thus different from that in the intestine (Kodicek, 1956). It may be of significance, that tissues intimately connected with the turnover of phosphate, such as bones, intestines and kidneys, contained significant amounts of the labelled vitamin D.

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Bacterial Oxidation of Fatty Acids at Alkaline pH. By A. T. H. BURNESS and H. K. KING. (*Department of Biochemistry, University of Liverpool*)

An unidentified organism (apparently *Micrococcus* sp.) isolated from commercial soap can use higher fatty acids as sole source of carbon and will oxidize these acids in the Warburg manometer. Growth (on nutrient broth with 1% starch) occurs over the pH range 7–11 (optimum pH 9). The optimum for oxidation of acetate is also pH 9, but oxidation is still rapid at pH 11. Stearate, oleate, and the *n*-C_6, C_8, C_9, C_{10}, C_{12}, C_{14} and C_{16} fatty acids are oxidized almost as rapidly as acetate. The following (in descending order) are less readily attacked; valerate, propionate, butyrate, lactate = glucose. Oxidation of higher fatty acids at pH values at which they exist as soaps is unusual, and this organism should be of value in the study of fatty acid oxidation.

We are indebted to Mr W. R. Fernell for a culture of this organism; to the Agricultural Research Council for a grant (to A.T.H.B.) and to the Medical Research Council for a grant towards apparatus and materials.

The Study of Internal Solutes of Mitochondria by Rapid Centrifugal Transfer to a Fixation Medium. By W. C. WERKHEISER and W. BARTLEY. (*Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, University of Oxford*)

In previous work (Bartley & Davies, 1954) it was recognized that changes in organic phosphate content of mitochondria might occur during the interval between sedimentation of the particles and addition of acid fixative, but reduction of this interval was then impossible. We now find that 20% of the endogenous organic phosphate of mitochondria sedimented from 0·25 M sucrose at 22° can be hydrolysed in the few minutes which necessarily elapse between sedimentation and fixation by the

original procedure. A reduction of this interval to less than a second has now been achieved by centrifuging mitochondria through a layer of water-immiscible silicone fluid into an aqueous fixative. With this procedure, breakdown of mitochondrial organic phosphate after centrifugation is completely eliminated.

The accurate analysis of internal solute concentrations in mitochondria further requires the estimation of the amount of such solutes contained in the extra-particulate fluid of the pellet. This fluid has been estimated from the distribution between pellet and medium of radioactive polyglucose previously added to the medium.

These methods have been used in a survey of the concentrations of the major intraparticulate solutes of rat liver mitochondria prepared at 0° in a variety of ways and suspended in a variety of media. For example, when the suspension medium was 0·25M sucrose the concentration (mMolar) in the mitochondrial water and the medium were respectively: K^+, 45, +1; Na, 4, 0·5; Cl^-, 16, 3·3; orthophosphate, 7, 0·1; organic phosphate, 38, 0·3; sucrose, 158, 231. On addition of NaCl and KCl, the values were: K^+, 87, 37; Na^+, 38, 33; Cl^-, 79, 67; orthophosphate, 7, 0·1; organic phosphate, 41, 0·2; sucrose, 173, 231. On the further addition of 0·1 vol. of a commercial sample of Na 'ATP' containing approximately equal amounts of ATP and ADP, the values were: K^+, 84, 34; Na^+, 55, 49; Cl^-, 77, 63; orthophosphate, 18, 1·3; organic phosphate, 46, 11; sucrose, 162, 216.

This survey of intramitochondrial solutes at 0° provides a basis for the interpretation of changes in solute distribution occurring with changes in metabolism.

One of us (W.C.W.) is a Research Fellow of the National Cancer Institute, U.S. Public Health Service.

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Investigations of the Chromatographic Separation of Urinary Gonadotrophins. By W. R. Butt, Joyce D. Ingram and Brenda P. Round. (*The United Birmingham Hospitals, Department of Clinical Endocrinology, Birmingham, and Midland Hospital for Women, Birmingham,* 11)

Gonadotrophins were prepared from the urine of pregnant (HCG) and postmenopausal (HMG) women by a modification of the method of Johnsen (1955) using Decalso F (Permutit Co. Ltd.) as adsorbent. The products were extracted by 60% ethanol in 10% ammonium acetate (with ammonia to pH 8·5) and then re-precipitated by increasing the concentration of ethanol to 85%. HCG contained about 1000 i.u. chorionic gonadotrophin per mg. (rat prostate assay) and the median effective dose of HMG when assayed by the mouse uterus method (Brown, 1955) was between 0·1 and 0·2 mg. The yield was 5–10 mg./1000 ml. urine which compares favourably with the values reported by Johnsen, but represents less total activity than is obtained by kaolin adsorption (Loraine & Brown, 1954).

The gonadotrophins were fractionated by chromatography on columns of tricalcium phosphate (Crooke & Butt, 1953). They were adsorbed more firmly than those prepared by the kaolin method. HMG was eluted in a solution of Na_2HPO_4 (molarity > 0·01) and HCG was eluted in 0·02M-Na_3PO_4. The best conditions for the separation of HMG and HCG were elution of HMG in 0·015M-Na_2HPO_4 followed by elution of HCG in 0·02M-Na_3PO_4 containing 0·1M-NaCl. The median effective dose of HMG was now reduced to 0·02 mg. (about 50 times as active per mg. as the best kaolin preparations).

The chemical properties of these fractions were investigated by the methods previously described for kaolin extracts (Butt, 1956). Qualitatively the preparations were quite similar to each other and to the corresponding kaolin extracts. They contained the common amino acids, glucosamine, galactose, mannose and fucose. HMG contained 12%, and HCG 2%, hexose (anthrone method). Calculation from the u.v. adsorption (Beaven & Holiday, 1952) gave tyrosine as (HMG first), 3·5, 3·0% and tryptophan 4·2, 3·2%.

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The Site of Action of Inhibitors of α-Chymotrypsin. By B. S. HARTLEY. (*Department of Biochemistry, University of Cambridge*)

We have investigated the effect of pH on the reaction of α-chymotrypsin with *p*-nitrophenyl diethyl phosphonate. This is a second-order reaction in which a group in the active centre is substituted and *p*-nitrophenol is liberated (Hartley & Kilby, 1952). We find that the effect of pH on the rate of reaction is consistent with the ionization of a single group of pK 6·6. Since the inhibitor contains no group ionizing at this pH, this pK must apply to a group in the enzyme. Since the reaction shows second-order kinetics, this group must control the initial combination with the inhibitor. These results are consistent with the hypothesis that organo-phosphorus inhibitors react initially with the imidazole ring of a histidine residue. Since the substituted phosphorus can be isolated attached to a serine hydroxyl group, it seems likely that an intramolecular transfer of the phosphoryl group, from histidine to serine, can occur (Schaffer, May & Summerson, 1953).

In previous studies on the substitution of groups in chymotrypsin with fluorodinitrobenzene, we concluded that the main effect was to increase the Michaelis constant of the enzyme, and that the maximum velocity was unaffected (Massey & Hartley, 1956). In these experiments, the enzyme activity was assayed using N-acetyl-L-tyrosine ethyl ester (ATEE) as substrate at pH 7·8, 25°, in the presence of 30% methanol. Under these conditions K_m for α-chymotrypsin is 0·03M and k_3, the maximum velocity constant, $7{\cdot}6 \times 10^3$ min.$^{-1}$. However, we have recently repeated these experiments using ATEE as substrate in 0·1M-NaCl, 0·001M versene pH 7·8, 25°, in the absence of methanol. Under these conditions K_m for α-chymotrypsin is 0·001M and k_3 is $8{\cdot}7 \times 10^3$ min.$^{-1}$. We find that the main effect of reaction of the enzyme with fluorodinitrobenzene is on the maximum velocity under these conditions, and that the Michaelis constant is unaffected. It seems possible that this discrepancy may be due to different steps in the catalysis being rate-limiting in the presence and absence of 30% methanol respectively. It has been suggested that the inhibition produced by reaction with fluorodinitrobenzene under the conditions of reaction may be due to substitution of a histidine residue (Massey & Hartley, 1956). If so, this substitution affects mainly the maximum velocity of the reaction rather than the Michaelis constant when the activities are measured under optimal conditions.

I thank Miss P. A. Lewis for technical assistance.

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Non-enzymic Synthesis of *N*-Methylindole under 'Physiological' Conditions. By J. HARLEY-MASON and A. A. P. G. ARCHER. (*Chemical Laboratory, University of Cambridge*)

It is widely accepted that anthranilic acid is a precursor of indole and hence tryptophan in several micro-organisms. It has been shown that in the addition of a (hypothetical) two-carbon fragment to anthranilic acid: (*a*) the carboxyl group is displaced as carbon dioxide (Nyc, Mitchell, Leifer & Langham, 1949); and (*b*) one of the two carbon atoms becomes attached to the benzene ring in the position where the carboxyl group was (Yanofsky, 1955*a*). Evidence has been presented for various precursors of the two-carbon unit, which are active in cell-free systems: (*a*) 5-phosphoribosylpyrophosphate (Yanofsky, 1955*b*); (*b*) hydroxypyruvic acid (Rafelson, 1955); and (*c*) pyruvate or α-ketoglutarate (Trudinger, 1956).

In extension of earlier experiments (Harley-Mason, 1955) we have now found that reaction of *N*-methylanthranilic acid with hydroxyacetaldehyde or hydroxypyruvic acid at room temperature in an aqueous buffer at pH 7 under nitrogen gives *N*-methylindole. No indole was, however, obtained when anthranilic acid was used and *N*-acetyl- and *N*-phospho-anthranilic acid were also ineffective. The non-enzymic synthesis of the indole ring-system under very mild conditions has thus been shown, and the bearing of these findings on indole formation *in vivo* will be discussed.

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The Reaction of Bromine with Pyrimidine Compounds. By W. E. Cohn. (*Agricultural Research Council Virus Research Unit, Molteno Institute, and Chemical Laboratory, University of Cambridge*)

The reaction of aqueous bromine with pyrimidine bases has been of classic utility in structural determinations, analysis, and in the preparation of derivatives. The formation of a saturated oxidized addition product as the first step, followed by heat-induced elimination reactions, has been the usual method of producing the mono-bromo derivatives (Wheeler & Johnson, 1907; Levene & LaForge, 1912). In the case of uracil, the first substance has been isolated and identified (Wheeler & Johnson, 1907). The possibility of diverting it to other ends has motivated a further investigation of its properties utilizing the techniques of ultraviolet spectrophotometry and chromatography.

The addition of aqueous bromine to pyrimidines or their nucleosides or nucleotides in neutral or slightly acid solution results in almost complete loss of ultraviolet absorption; that which remains ($<10\%$) is neither that of the original nucleotide nor of the second product (e.g. bromouridylic acid). Two molcs of Br_2 are required to achieve the minimum of ultraviolet absorption, which is ascribed, as formerly, to the saturation of the carbon-carbon double bond (4, 5 in the classical nomenclature, 5, 6 in the newer). Boiling of this first product at acid pH values results in the gradual reappearance of ultraviolet absorption spectra corresponding to those published for the second products (Roberts & Visser, 1952; Beltz & Visser, 1955). The double bond is regenerated in this process.

The first (saturated) product, if treated briefly with dilute alkali at room temperature, is no longer capable of being converted to the second (unsaturated) product; furthermore, nucleosides or nucleotides now give quantitative orcinol reactions, in contrast to the saturated compounds. The lability of dibromopyrimidine and pyrimidine diglycol rings to alkali has been noted (Wheeler & Johnson, 1907; Baudisch & Davidson, 1925; Soodak, Pircio & Cerecedo, 1949). Presumably the acyclic riboside or ribotide which is produced by alkali is easily split by acid, liberating ribose or ribose phosphate which gives the usual orcinol reaction. This would be analogous to the behaviour of dihydrouridylic acid (Doherty & Cohn, 1956). It has also been observed that substances corresponding in history and spectrum to bromouridylic and bromocytidylic acids react with orcinol in a quantitative manner.

These observations have significance with respect to improvement of assay procedures for pyrimidine nucleosides and nucleotides involving the use of bromine (Massart & Hoste, 1947; Soodak *et al.* 1949) and by ultraviolet spectrophotometry.

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'Complete' and 'Incomplete' Grass Pollen Antigens in Relation to Skin Reactivity and other Pollen Constituents. By R. Augustin. (*Wright-Fleming Institute, St Mary's Hospital, London, W. 2*)

Grass pollen antigens are of particular interest in asthma and hay fever. In the human they give rise to two types of non-precipitating antibodies, one of them the tissue-sensitizing reagin. In rabbits and guinea pigs precipitating antibodies are formed. We still do not know whether the same antigens are responsible for all three types of antibody formation (Augustin, 1955).

The protein conjugates in crude pollen extracts, responsible for the histamine-like skin reactions in hay-fever subjects, are not eluted from the base-line position with the developing fluids usual in paper chromatography (Augustin, 1953). Their carbohydrate component will be shown to consist of arabinose and galactose, and possibly glucose in some pollen extracts.

By means of Ouchterlony plates, grass pollens can be shown to be antigenically related, but not all the pollens share the common main antigen, e.g. not bermuda, spartina or maize. A quantitative double-gel diffusion method (Augustin & Hayward, 1955) enabled us to investigate further

the nature of the common main antigen in its relation to skin reactivity.

Salt fractionation at acid pH yields a number of biologically highly active proteins, their electrophoretic mobility depending upon pigment and carbohydrate content (Augustin, 1953). Ability to precipitate with rabbit antibody is largely destroyed during this process. Formation of biologically active fragments (and conjugates?), capable of inhibiting antigen-antibody precipitation with the intact molecule, takes place during heating of extracts and other chemical interference. The sugar-rich molecules formed during heating are non-dialysable. The term 'incomplete antigens' is therefore used in preference to 'haptens'.

Dissociation of skin reactivity from the capacity to precipitate antibody has also been achieved by purely physical methods, such as differential extraction of pollens and electrophoresis. Relations with other biological systems (Bawden & Kleczkowski, 1941; Wagman, 1954) will be discussed.

The effects of proteolytic and carbohydrate splitting enzymes on skin activity versus gel diffusion activity will be described.

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The Biosynthesis of Guanosine 5′-Monophosphate by Pigeon Liver Preparations. By N. L. Blumsom (introduced by G. Weber). (*Department of Biochemistry, University of Sheffield*)

Greenberg (1951) and Schulman & Buchanan (1952) showed that incubation of 4-amino-imidazole 5-carbonamide, [^{14}C]formate and ribose phosphate with pigeon liver homogenate led to the formation of inosinic acid (IMP).

It was decided to use this system, with the addition of adenosine triphosphate (ATP), glutamine and aspartic acid as extra substrates, to investigate the biosynthesis of the adenosine phosphates. After incubation, in phosphate buffer pH 7·2 at 37°, the barium salts of the nucleotides were isolated (see Umbreit, Burris & Stauffer, 1951) converted to the sodium salts and separated on paper chromatograms by the two-solvent method of Krebs & Hems (1953). The nucleotide spots were cut out and the radioactivity counted directly (Greenberg, 1951). Radioactivity was found to be associated with the IMP and ATP areas.

It was found that a phosphate buffer extract of acetone-dried pigeon liver with the addition of an aqueous boiled extract of fresh liver could replace the homogenate in this synthesis and that ammonium carbonate could replace glutamine and aspartic acid.

The radioactive material from the ATP area was quantitatively eluted and then chromatographed in *n*-butanol-acetic acid (Partridge, 1948). The radioactivity appeared in the IMP/AMP/GMP areas (GMP = guanylic acid; AMP = adenylic acid).

Addition of myokinase to the reaction mixture did not result in a redistribution of the counts in the adenosine areas, providing further evidence that the radioactivity in the ATP area was due to another compound. Guanosine compounds were an obvious possibility for consideration and it was found that GMP ran in the ATP area following the Krebs & Hems (1953) procedure.

The labelled compound which ran in the IMP/AMP/GMP area in *n*-butanol-acetic acid was hydrolysed with snake venom, which contains a specific 5′-nucleotidase, as described by Johnson, Kaye, Hems & Krebs (1953) and rechromatographed in the same solvent. The radioactivity appeared in the guanosine area. Paper electrophoresis at pH 2·8 in 0·02 M ammonium formate confirmed that the material was guanosine.

These results show that *in vitro* synthesis of guanosine 5′-monophosphate takes place when 4-amino-imidazole 5-carbonamide and ribose 5-phosphate are incubated with a pigeon liver preparation.

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Preparation of a Purified Feather Keratin Monomer. By A. M. Woodin. (*M.R.C. Ophthalmological Unit, Institute of Ophthalmology, London, W.C.* 1)

Feather keratin extracted by solutions of urea and reducing agents, and subsequently oxidized with performic acid, is converted to cysteic acid-keratin, a protein with a small distribution of particle size and a molecular weight of 10 000 (Woodin, 1954). Cysteic acid-keratin contains only one-tenth of a molecular proportion of free α-amino groups (Woodin, 1955); the reaction with carboxypeptidase and hydroxyl ion is consistent with the absence of free α-carboxyl groups, and it has been concluded that the feather keratin monomer is an unbranched cyclic polypeptide (Woodin, 1956). The slight reaction with reagents capable of detecting end-groups has been ascribed to impurities.

Cysteic acid-keratin prepared from feathers which have been extracted with 10M urea before solubilization contains fewer of these impurities, and the properties of this material supports the cyclic polypeptide hypothesis. However, it is possible that the monomer consists of a collection of cyclic polypeptides of similar size. To obtain a product of which the chemical homogeneity could be assessed, the action of performic and peracetic acids on the amide groups and tyrosine has been studied.

Before oxidation, soluble feather keratin contains 10 equivalents of amide groups and 1 equivalent of tyrosine in a unit of weight 10 000. Tryptophan is absent. After oxidation with performic acid at 0° for 8 hr., followed by dialysis, only 10% of the amide groups and 25% of the tyrosine can be recovered. Oxidation of the disulphide bonds with peracetate at pH 4 is very slow, and after 5 hr. only 50% of the tyrosine can be recovered. Quantitative oxidation of the disulphide bonds with quantitative recovery of the amide groups and tyrosine is obtained by trea ment with performate at pH 1·0 at 5° for 20 min.

The solubility properties of cysteic acid keratin prepared in this way are not those of a homogeneous substance, but it is possible that some of the heterogeneity is due to the adoption of different configurations by the same molecule.

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Some Aspects of the Interaction of Sulphydryl and Acid Imino Groups with Formaldehyde. By S. Lewin. (*Physical Chemistry Laboratory, South-West Essex Technical College, London, E.* 17)

Sulphydryl groups react reversibly with >C=O groups to form semimercaptals or semimercaptols, thus

$$R_1SH + R_2{-}\overset{\overset{O}{\|}}{\underset{R_3}{C}} \rightleftharpoons R_1{-}S{-}\overset{OH}{\underset{R_3}{C}}{-}R_2. \qquad (1)$$

Acid imino groups react similarly to form the corresponding hydroxymethyl compounds, thus

$$R_4R_5NH + HCHO \rightleftharpoons R_4R_5N.CH_2OH \qquad (2)$$

and the products in (1) and (2) may react with another containing a sulphydryl or imino group, with the elimination of water.

The reaction of basic amino or imino groups with formaldehyde (the Sorensen titration, for references see French & Edsall, 1945) or sugars (for references see Frankel & Katchalsky, (1938, 1941), Lewin *et al.* (1956), Danhey & Pigman (1951)) is accompanied by pH depressions owing to the imposition of the reversible combination on the acid-base equilibria initially solely present, e.g.

$$-NH_3^+ \rightleftharpoons -NH_2 + H^+$$

$$-NH_2 + HCHO \rightleftharpoons -NH.CH_2.OH$$

which may be accompanied by a second carbonyl group reaction.

Acid imino groups and sulphydryl groups being acidic, we may predict that the imposition of the reversible reaction with formaldehyde on the acid-base equilibria present should result in a pH elevation according to the scheme

$$-XH \rightleftharpoons -X^- + H^+ \quad \text{(where X = N or S)}$$

$$-XH + HCHO \rightarrow X.CH_2OH.$$

Hence, titrations with alkali, in presence and absence of formaldehyde, were carried out on such compounds (e.g. succinimide, hydantoin, uracil, mercaptoethanol), and pH elevations were noted in these reactions. pH elevations have already been noted by Pirie & Pinhey (1929) in the glutathione–formaldehyde interaction and by Birch & Harris (1930) in the formaldehyde–cysteine interaction at the higher pH ranges, but the significance of the interactions was apparently not appreciated.

In all the cases examined, the pH elevations were found to be independent of the initial pH at low formaldehyde concentrations, and were interpretable in terms of interaction of one –XH with one molecule of formaldehyde. The decrease in the value of the equilibrium constants with temperature showed the reaction to be exothermic.

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The Application of the Formaldehyde Titration to the Assignment of p*K* values in Compounds Containing Acid Groups such as –NH or –SH and Basic Groups such as $-NH_2$ or –NH.

By S. Lewin. (*Physical Chemistry Laboratory, South-West Essex Technical College, London, E.* 17)

The assignment of p*K* values in compounds containing two groups such as $-NH_2$ and –SH or $-NH_2$ and acid –NH when the p*K* values are comparatively close is fraught with difficulties, and arguments involving comparison with the p*K* values of series of compounds having similar groups have been used to determine the allocation of such values and even to reverse values allocated on other grounds. Thus, Birch & Harris's (1930) allocation of the p*K* values of *ca.* 8·3 and *ca.* 10·8 for the $-NH_3^+$ and –SH groups in cysteine, and a parallel assignment of the higher p*K* value for the –SH group in glutathione by Pirie & Pinhey (1929) have been inverted by Peters (1947) and by Calvin (1954).

However, the findings (Lewin, 1956) that the reactions of acid imino and –SH groups with formaldehyde result in a pH elevation are in distinct contrast with the pH depression resulting from the reaction of basic amino groups with formaldehyde. These results can be used as criteria for the assignment of p*K* values, since in the buffering range of a basic amino group a pH depression should be obtainable on titration in presence of formaldehyde, and on the acid –NH or –SH range a pH elevation should occur. This criterion has been applied to various compounds. For example, in adenine, the formaldehyde titration resulted in a pH depression on the acid side, and a pH elevation on the alkaline side of neutrality, thus agreeing with the assignment (Taylor, 1948) of the p*K* values in the regions of *ca.* 4·15 and *ca.* 9·8 to the $-NH_3^+ \rightleftharpoons -NH_2 + H^+$, and $-NH \rightleftharpoons N^- + H^+$, respectively. In the case of cysteine, however, the pH depression obtained at the lower pH range, and the pH elevation obtained at the higher pH range (p*K* *ca.* 10·6) appear incompatible with French & Edsall's (1945), Peters's (1947) and Calvin's (1954) inversions; and, pending further considerations, the initial assignments of the highest p*K* values to the –SH group should be favoured.

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DEMONSTRATIONS

An Improved Filtering Device Specially Useful for Radioactive Deposits. By W. M. DALE and C. W. GILBERT. (*Departments of Biochemistry and of Physics, Christie Hospital, and Holt Radium Institute, Manchester*)

Faced with the problem of quantitatively separating suspensions of radioactive sulphur in a highly dispersed semi-colloidal state from a concentrated aqueous thiourea solution, it was necessary to construct a new filter device. Existing sintered-glass filters of fine porosity (no. 5) failed because filtration was very slow and incomplete, through early clogging of pores. A new filtration device was therefore developed which made filtration fast and efficient.

The new unit has the advantage over existing filters in pressing together one or more filter disks at their circumference firmly, evenly and strictly vertically without shearing action, leaving the rim free of deposit. The speed and efficiency of filtration can easily be controlled by a suitable choice and number of filter disks and the use of 'Supercel'. Subsequent counting of radioactive deposit, where necessary, can be carried out on the individual filter disks after dismantling the filter.

The filter consists of four parts: a funnel and filter stem of stainless steel and two clamping rings. A slot in the funnel engages with a spigot in the lower ring preventing a shearing action on the filter-paper disks when tightening the clamping rings with spanners applied to them.

Cleaning and removal of radioactivity is much easier than with sintered-glass filters. The fact that the clamping rings are separated from the funnel and filter stem is a further aid to cleaning.

The Capillary Tripod Circular Chromatography Dish. By E. KAWERAU. (*Department of Chemical Pathology, St Mary's Hospital, London, W. 2*)

The apparatus has been made in two sizes and consists of specially cast shallow desiccator dishes of 15 and 26 cm. diameter. The paper circle is held in position between the flanges of the base and the lid, and is supported in the centre by a capillary tube mounted on a tripod. The capillary tube, of standard bore 0·36 mm., reaches almost to the floor of the dish, and by capillary ascent feeds the centre of the paper disk. The legs of the tripod locate with a circular groove cut in the centre of the dish and this device assures that the tripod does not become dislodged during the running of the chromatogram. The aim in designing this piece of apparatus has been to achieve some measure of standardization. The small dish has been found useful in routine urine analysis and student teaching. The large dish can replace the tank for most unidimensional work.

A Versatile Constant-Temperature Water Bath and Shaking Device for the Incubation of Small Amounts of Tissue in any Gas (Modified Dubnoff Shaker). By J. M. LEYTHAM and P. J. RANDLE. (*Department of Biochemistry, University of Cambridge*)

The metabolism of isolated tissues has frequently to be studied with a gas phase of given composition even though measurement of gas exchange is not required. The use of manometric equipment in such instances is unnecessarily expensive and space-consuming, particularly when observations on a large number of tissue samples are required. An apparatus, suitable for this purpose, and avoiding the use of manometers has been described by Dubnoff (1948), and the apparatus shown is a modification of this.

The apparatus consists of a general purpose water bath fitted with temperature control, and an enclosed tray which may be shaken in the bath at 60–140 cycles/min. The tissue samples may either be incubated in small beakers, in which case the gas is circulated through the hood holding the tray, or alternatively incubated in small conical flasks previously gassed and sealed with rubber bungs. With the present design, which is commercially available, up to forty samples of tissue may be incubated in a very small space.

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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **353rd Meeting** *of the Biochemical Society was held in the Department of Biochemistry of the University of Leeds on Friday,* 13 *July* 1956, *at* 11 *a.m., and took the form of a Symposium on* 'Metals and Enzyme Activity'.

COMMUNICATIONS

Enzyme-Metal-Substrate Complexes as Co-ordination Compounds. By L. E. ORGEL. (*Department of Theoretical Chemistry, University of Cambridge*)

The possible functions of metal ions in enzyme systems are classified according to the nature of the groups present in the first co-ordination sphere of the metal. It is suggested that, although both protein and substrate are attached to the metal ion in many and perhaps most metal-enzyme systems, there are a number of ways in which metal-protein interaction can influence reactivity in the absence of metal-substrate bonding. The possibility of metal-substrate bonding without metal-protein attachment is also considered briefly.

The characteristic properties of the functional groups of amino acids as co-ordinating groups are considered in some detail. The affinity of different groups and configurations of side chains for the metals commonly present in biological material are deduced by analogy with related inorganic systems. Enzyme-metal-substrate molecules are also discussed in the same general way.

Finally the mechanisms of certain metal enzyme catalysed reactions, for example decarboxylation and oxidation reactions, will be discussed in the light of similar reactions which are known to occur in simple systems.

The Nature of Metal-Peptide Complexes in Aqueous Solution and their Relation to Proteolytic Activity. By B. R. RABIN. (*Department of Biochemistry, University College, London, W.C.* 1)

In the formation of complexes of dipeptides with the ions Co^{2+}, Mn^{2+} and Cu^{2+}, interactions occur at the free amino group and one or other of the groups associated with the peptide bond. Evidence is available that the latter site is the peptide oxygen atom in the cobaltous and manganous chelates; this is also true for the cupric complexes at low pH values. At higher pH values, ionization of the peptide hydrogen atom can occur from the cupric complexes ($pK = 4{\cdot}25$ at 25°) and a rearrangement is possible to produce complexes involving co-ordination of the peptide nitrogen atom. The nature of the complexes formed and the factors affecting their stability will be discussed.

Many proteolytic enzymes are activated by metal ions and it has been postulated that the role of the metal in these systems is to mediate the binding of the substrate to the enzyme, forming a mixed enzyme-metal-substrate complex. Hypotheses have been advanced concerning the nature of the catalysis in these enzymes; these speculations will be discussed in the light of the data available concerning the sites of co-ordination in peptide-metal complexes and the possible mechanisms of hydrolysis of the peptide bond.

Univalent Metals and other Loose or Non-specific Activations. By F. C. HAPPOLD and R. B. BEECHEY. (*Department of Biochemistry, University of Leeds*)

The metal-enzyme complexes can be differentiated from the metalloenzymes by certain characteristics, most of which centre around the lower association constant of the metal with the apoenzyme. The work and reviews of Emil Smith and his colleagues (1954) on the peptidases, of Calvin (1954) and many others, give a picture of 'a promised land' not easy to explore and to know but from which significant knowledge is emerging and to which increasing explorations will be made. Malmstrom (1954) in his work on enolase has indeed studied the stability and the stoicheiometry of the interaction of metal and enzyme in relation to function in the metal-enzyme complex, but for obvious reasons of difficulty there have been few studies of this kind. This is in contrast to the clearer picture in the metalloenzyme field.

Physiologists have for long been aware that the distribution of K^+ and Na^+ as between the tissue cells and the extracellular fluids could be associated

with certain manifestations of physiological activity; as for example in the transport of glucose into and out of the cells, and on the transport of the amino acids.

Variations in the external concentration of pyridoxal also effects the transport of glycine with ascites tumour cells (Christensen, 1955), whilst pyridoxal phosphate is the coenzyme of a great number of enzyme systems which are associated with amino acid metabolism. The fact that K^+ apparently activates some of these enzyme systems may also represent a causal relationship between these facts and the protein metabolism of the cell.

That K^+ apparently activates a great number of enzyme systems, most of them associated with phosphate transfer, is also of significance as the work of Nachmansohn & John (1945), Black (1951), Stadtman (1952), Kackmar & Boyer (1953), Pressman & Lardy (1952), Muntz (1953), Von Korff (1953) and Von Korff, MacPherson & Glaman (1954) have shown. Ammonium and rubidium ions are also effective in some cases. This work will be discussed. The apparent activation of one of the pyridoxal phosphate enzyme systems, tryptophanase, by NH_4^+, K^+ and Rb^+ has been studied by Happold & Struyvenberg (1954) and this study extended by Beechey & Happold (unpublished). The following postulates have been critically examined: (1) that K^+ or NH_4^+ or Rb^+ are a part of the enzyme system, (2) that they are engaged in anchoring the substrate or coenzyme to the active centre, (3) that they accelerate the rate of breakdown of the enzyme substrate complex, and (4) that they affect the maintenance of the enzyme in an active form.

Evidence will be given to support postulate (4) and to exclude the other three.

The effect of monovalent cations on other pyridoxal phosphate enzyme systems has been studied in association with Saran & Hearfield.

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The Role of Metals in Oxidations Catalysed by Xanthine Oxidase and by other Metalloflavoproteins. By F. Bergel and R. C. Bray. (*Chester Beatty Research Institute, Royal Cancer Hospital, Fulham Road, London, S.W.* 3)

A brief review of flavoproteins will be given, with particular reference to those containing metals. The properties of xanthine oxidase (Avis, Bergel & Bray, 1955, 1956; Avis, Bergel, Bray, James & Shooter, 1956), which has been obtained in a purer state than any of the other metalloflavoproteins, will be mentioned as an example of this class of enzymes. The actual way in which molybdenum and iron function in the catalytic activities of this compound and the mechanism of oxidation of its substrates is largely unknown so far. However, some indications may be obtained from the dependence of full enzymic potencies on molybdenum, on the presence of stabilizers and on the absence of other heavy metals. As in this case the metal cofactors are not easily detached from the apoenzyme, other evidence as to their function will be deduced by analogy with metalloflavoproteins, such as nitrate reductase (Nicholas & Nason, 1954; Nicholas & Stevens, 1955), DPNH-cytochrome-*c* reductase (Mahler & Elowe, 1954), etc. Such speculations as to the mode of electron transport in these enzymes have been made by Mahler and others (Mahler & Green, 1954; Mahler, Fairhurst & Mackler, 1955; Mahler, 1955).

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Catalytically Active Haemoproteins, with Special Reference to the Cytochromes. By E. C. SLATER. (*Laboratory of Physiological Chemistry, University of Amsterdam, The Netherlands*)

There are three types of catalytically active haemoproteins in the living cell.

(1) *Catalase and peroxidase.* Catalase and peroxidase are haematin compounds (ferric iron) in which the iron atom is combined with the four pyrrole nitrogen atoms, with a nitrogenous group of the protein and with water. Hydrogen peroxide or alkyl hydrogen peroxides are able to oxidize the haematin to an intermediate, which is reduced back to the ferric compound by certain hydrogen donors, which are thereby oxidized. Catalase reacting with hydrogen peroxide is a special case, in which H_2O_2 reacts both as the oxidizer of the haematin and as the hydrogen donor, yielding H_2O and O_2 respectively from the two reactions. Catalase and peroxidase are not readily reduced to the haem compound (ferrous iron).

(2) *Non-autoxidizable cytochromes.* In the cytochromes, all the six co-ordination places on the iron atom are combined with nitrogen (four with the pyrrole nitrogens, two with protein nitrogens). They are readily reduced to the ferrous state (haemochromogens), showing sharp absorption bands in the visible spectrum, and oxidized to the ferric compounds (parahaematins). Higher oxidation states than the ferric are not known. With rare exceptions, treated in the next group, the ferrous compound is not rapidly oxidized by molecular oxygen, nor does it combine with carbon monoxide. The ferric forms do not readily combine with cyanide or other respiratory inhibitors.

(3) *Autoxidizable cytochromes.* The most important member of this group is cytochrome a_3, which is the only component of the mitochondrial respiratory enzyme systems which reacts with oxygen. It is a parahaematin which is reduced by the electron–transferring system of the respiratory chain to the haemochromogen, and is reoxidized by molecular oxygen. It is not known whether oxidation states higher than ferric are involved in this reaction. The ferric form combines firmly with cyanide and other respiratory inhibitors, while the ferrous form combines with carbon monoxide. Cytochrome b_5, found in microsomes, also appears to be somewhat autoxidizable. In some microorganisms, in which cytochrome a_3 is absent, it is possible that cytochrome a_2 is the component which reacts with oxygen.

In mitochondria and in active preparations of the respiratory chain prepared by the fragmentation of mitochondria, cytochromes b, c, c_1, a and a_3 are all present, firmly bound to the same particle. They must be linked to the particle in such a way as to promote the rapid transfer of electrons through the iron atoms of the cytochromes to cytochrome a_3 and thence to oxygen. Nevertheless, each must be regarded as separate molecules, rather than as different prosthetic groups of a single molecule (Green), since the degree of oxidation and reduction in the aerobic steady state of each cytochrome is different, and can be changed in opposite directions by the addition of inhibitors.

[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of Papers read are published as received from the authors.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **354th Meeting** *of the Biochemical Society was held in the Department of Biochemistry and Pharmacology of University College, Dublin, on Friday,* 14 *September, and in the Department of Biochemistry, Trinity College, Dublin, on Saturday,* 15 *September* 1956, *starting at* 10.30 *a.m. on Friday,* 14 *September. The following papers were read:*

COMMUNICATIONS

Purine Metabolism and Riboflavin Synthesis in *Eremothecium ashbyii*. By E. G. Brown, T. W. Goodwin and O. T. G. Jones. (*Department of Biochemistry, The University, Liverpool* 3)

It is now established that purines stimulate flavinogenesis in *Eremothecium ashbyii* by being incorporated into the riboflavin molecule (Goodwin & Pendlington, 1954; McNutt, 1954), but the pathways involved are unknown. The metabolism of various purines by *E. ashbyii* has now been studied. With adenine after 5 days incubation some (A) remained unchanged, some (B) was converted into hypoxanthine, and the remainder (C) could not be accounted for as purine (no xanthine was ever detected). The incorporation of adenine into riboflavin accounted for some but never all of fraction C. The proportions of these fractions varied with the initial adenine concentration; for example, with an initial concentration of 3 mg./100 ml. the relative proportions were: A, 10·5%; B, 14·7%; and C, 74·8%; whilst with a concentration of 10 mg./100 ml., the values were 21·9, 37·0 and 41·1%, respectively.

Adenosine behaved similarly, part being converted into inosine; fraction C was, however, much greater although riboflavin synthesis was the same as with an equivalent amount of adenine.

Under the same conditions hypoxanthine, xanthine and guanine gradually disappeared from the medium without producing detectable amounts of other purines. The riboflavin produced accounted for some but not all of the metabolized fraction.

When examined for flavinogenic activity, guanine, xanthine, adenine and hypoxanthine fall into that order of decreasing effectiveness. This observation, together with those just quoted, give no indication that xanthine (the purine immediately related to rings B and C of riboflavin) is an obligatory intermediate in the conversion of purines into riboflavin. As adenine is more active than hypoxanthine, it is probable that adenine is incorporated into riboflavin via two pathways, one of which does not involve hypoxanthine.

The effect of a number of azapurines on flavinogenesis has also been examined. Azaxanthine and aza-adenine inhibit flavinogenesis more than growth, the lowest effective concentrations being 4 and 0·125 mg./100 ml. respectively. Azaguanine, however, at 0·25 mg./100 ml. inhibits both growth and flavinogenesis equally. Azahypoxanthine is comparatively inactive, inhibiting growth and flavinogenesis only slightly at 20 mg./100 ml.

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The Formation of Tyrosol (2-*p*-Hydroxyphenylethanol) from Tyrosine by *Saccharomyces cerevisae*. By S. SentheShanmuganathan (introduced by J. L. Peel). (*A.R.C. Unit for Microbiology and Department of Microbiology, Sheffield University*)

Ehrlich (1907) first showed that cultures of yeast growing in presence of fermentable carbohydrates convert amino acids into alcohols containing one less carbon atom than the parent amino acid. This reaction has been investigated further using tyrosine as substrate and *Saccharomyces cerevisae* T 35 as the organism, and evidence in support of the following reaction chain (cf. Harden, 1932) has been obtained:

$$\text{Tyrosine} \xrightarrow{-[NH_2]} p\text{-hydroxyphenylpyruvic acid} \xrightarrow{CO_2} p\text{-hydroxyphenylacetaldehyde} \xrightarrow{2H} \text{tyrosol}$$

In contrast to tyrosine and *p*-hydroxyphenylpyruvic acid, tyrosol and *p*-hydroxyphenylacetaldehyde are extracted into ether from alkaline solutions. The metabolism of the first two compounds can therefore be followed by measuring the appearance of ether-extractable phenol (EEP), the phenol so extracted being estimated colorimetrically by the method of Folin & Denis (1915). *p*-Hydroxyphenylacetaldehyde and tyrosol in ether extracts can be distinguished by the bisulphite-binding reaction.

When whole washed cells were incubated with tyrosine plus glucose at 25° and in M/15 phosphate buffer pH 6·0, tyrosol was produced. No tyrosol was formed in the absence of glucose. At pH 6·2, cell-free extracts of the organism prepared by crushing in a bacterial press (Hughes, 1951) and then extracting with M/15 phosphate buffer pH 6·2, converted tyrosine to EEP of which 90% was the aldehyde. The reaction required pyridoxal phosphate as cofactor and 2-oxoglutarate as amino acceptor, glutamic acid being formed in an amount equivalent to the amount of tyrosine disappearing (cf. the work of Bigger-Gehring (1955) on transamination in *S. fragilis*). *p*-Hydroxyphenylpyruvic acid was completely decarboxylated by the same extract to give the same EEP products as tyrosine. This reaction was used to obtain preparations of *p*-hydroxyphenylacetaldehyde for use as substrate in subsequent experiments, as the compound could not be obtained pure by chemical means.

The transfer of hydrogen from reduced DPN to the aldehyde has been shown spectrophotometrically. Only a partial reduction of the aldehyde to tyrosol could be obtained in cell-free extracts with hexosediphosphate as the hydrogen donor, but a complete reduction was observed with whole cells in presence of glucose.

These results suggest that whole cells form tyrosol from tyrosine by the scheme postulated above, the amino acceptor and hydrogen donor being derived from glucose.

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The Origin of the Carbon Atoms of Cholesterol. By J. W. Cornforth, Irene Youhotsky-Gore. (*National Institute for Medical Research, Mill Hill, London, N.W.* 7) and G. Popják. (*M.R.C. Experimental Radiopathology Research Unit, Hammersmith Hospital, London, W.* 12)

Methods have been worked out for the chemical degradation of the ring-structure of cholesterol in order that the origin of its carbon atoms from [^{14}C]acetate may be determined. Previous work established the pattern of distribution of acetate methyl and carboxyl carbons in the side chain (Würsch, Huang & Bloch, 1952), in ring A (Cornforth, Hunter & Popják, 1953*a*, *b*) and in part of ring B (Cornforth *et al.* 1953*b*; Bloch, 1953; Dauben & Takemura, 1953). Woodward & Bloch (1953) concluded that C-13 and the angular methyl carbon C-18, attached to it are derived from the methyl carbon of acetate.

By the application of new methods to two samples of [^{14}C]cholesterol, biosynthesized by rat liver slices from $CH_3{}^{14}COOH$ and $^{14}CH_3COOH$ respectively, it was determined that carbons 8, 11, 12, 14 and 16 originate from the carboxyl and carbons 9, 15 and 17 from the methyl carbon of acetate. The origin of C-13 and C-18 from the methyl carbon of acetate was also confirmed by an independent method.

Thus the pattern of distribution of acetate carbons in the entire cholesterol molecule is known. This pattern agrees with the 'squalene hypothesis' of the biogenesis of cholesterol (Woodward & Bloch, 1953) and with the pattern of distribution of acetate carbons in squalene biosynthesized from acetate (Cornforth & Popják, 1954). It is of interest to note that in ergosterol, which is also biosynthesized from acetate (Ottke, Tatum, Zabin & Bloch, 1951) carbons 11 and 12 come from the carboxyl carbon of acetate (Dauben & Hutton, 1956) just as in cholesterol. The squalene hypothesis demands that these two carbon atoms correspond to the two central carbons of squalene, which are known to be derived from the carboxyl carbons of acetate.

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The Effect of Vitamin A Deficiency on the Activity of the Liver Succinoxidase System in the Rat.

By E. R. REDFEARN. (*Department of Biochemistry, The Queen's University, Belfast, N. Ireland*)

An investigation is being made of the activities of certain enzymes in the vitamin A-deficient rat in the hope that it may help towards explaining how vitamin A acts in the body.

Weanling rats were grouped in pairs of the same litter and sex and were pair-fed on a vitamin A-free diet, the control animal of each pair receiving in addition 2250 i.u. vitamin A per week. When the vitamin A-deprived animals were showing the usual signs of deficiency (arrested growth and xerophthalmia) liver homogenates were prepared for the manometric estimation of endogenous respiration and succinoxidase (Potter, 1945) and for a spectrophotometric determination of succinate-cytochrome *c* reductase. Results of the manometric experiments are expressed as μl. O_2/mg. N/hr. and those of the spectrophotometric method as Δ log [cytochrome *c*]/mg. N/min., calculated by the method of Cooperstein, Lazarow & Kurfess (1950). The mean values $\pm$ S.E.M. are given, followed by the number of animals in each group.

It was found that the liver endogenous respiration of the A-deficient animals ($15{\cdot}2 \pm 0{\cdot}39$; 17) was significantly higher ($P < 0{\cdot}01$) than that of the control group ($12{\cdot}9 \pm 0{\cdot}69$; 14). This result is in line with the recent work on the endogenous respiration of isolated diaphragm and oxygen uptake of the whole rat in vitamin A deficiency (Blaizot & Serfaty, 1955; Blaizot & Bénac, 1955).

Although the mean activity of succinoxidase in the A-deficient group (603 ± 28; 18) was slightly higher than that of the controls (570 ± 34; 15) the difference was not statistically significant.

A marked difference ($P < 0{\cdot}001$), however, was found in the activities of cytochrome *c* reductase; the mean value of the A-deficient group ($1{\cdot}09 \pm 0{\cdot}02$; 12) was only 59% of that of animals receiving vitamin A ($1{\cdot}85 \pm 0{\cdot}17$; 9).

Recent reports (Nason & Lehman, 1955; Martius, 1954) suggest that other fat-soluble vitamins (E and K) are implicated in oxidative enzyme processes. It is possible that vitamin A (and other fat-soluble vitamins) may be concerned with preserving the structural integrity of enzyme systems. In this connexion it is interesting to note that Sheldon & Zetterqvist (1956) have observed an altered morphology of mitochondria from vitamin A-deficient mice.

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Biosynthesis of Rat Pancreatic Ribonuclease.

By VALDA M. CRADDOCK and C. E. DALGLIESH. (*Postgraduate Medical School, Ducane Road, London, W.* 12)

Most investigations of the biosynthesis of mammalian protein, using incorporation techniques, have involved studies of the uptake of radioactive amino acids into mixtures of proteins. This approach is limited, as the rate of incorporation into different components of the mixture may vary. To investigate the time of biosynthesis of a single protein molecule (cf. Dalgliesh, 1953) it is necessary to study incorporation into a single homogeneous molecular species.

We have established that, on the small scale appropriate to experiments with rat pancreas, ribonuclease (RNase) can conveniently be separated from amino acids and other proteins by ion-exchange chromatography, and the ^{14}C-radioactivity can be measured by 'infinite-thinness' counting techniques. Sliced pancreases of adult female rats were incubated in Krebs–Ringer bicarbonate containing unlabelled casein hydrolysate together with small amounts of a mixture of biosynthesized uniformly ^{14}C-labelled aliphatic amino acids (Dalgliesh & Dutton, 1956). Incorporation of activity into RNase occurred, though without net increase in RNase content. A large preparation of highly radioactive RNase was hydrolysed, the amino acids separated by chromatography, and their activities studied by autoradiography; the resultant pattern resembled that given by the free amino acids in the incubation medium. As all amino acids were incorporated, it was considered probable that incorporation had occurred by synthesis, rather than by exchange.

The rate of incorporation varied markedly with the purity of the amino acid mixture. Mixtures of aliphatic amino acids, prepared by starch chromatography (for removal of aromatic amino acids:

Moore & Stein, 1949) of hydrolysates of labelled yeast or *Chlorella* protein, were incorporated at comparable rates. Preparative paper chromatography of the crude amino acids left brown radioactive material on the origin. The purified amino acids were incorporated at about one-fifth the rate of the original mixture, whilst the radioactive material eluted from the origin of the preparative chromatograms was incorporated twice as fast as the original mixture. The evidence suggests that the stimulating factor contains radioactivity which is incorporated, so that it is likely to be an amino acid derivative. It may be similar to the carbohydrate derivatives of amino acids, which stimulate incorporation into reticulocyte protein (Borsook, Abrams & Lowy, 1955).

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The Concentration of the Cation Carrier in the Yeast Cell Wall as Milliequivalents per Kilogram of Centrifuged Yeast. By P. F. Duggan and E. J. Conway. (*Biochemistry Department, University College, Dublin*)

There is present in the yeast cell wall an active cation carrier which can actively transport the various inorganic cation species during fermentation but with very different affinities (Conway & Ryan, unpublished observations; Conway, 1954). Apart from H^+ ions, the affinity for K^+ ions is by far the greatest, being about 25 times greater than for Na^+ ions and a few hundred times greater than for Mg^{2+} ions.

The concentration of the carrier was measured from the amount of potassium instantaneously adsorbed by it and its subsequent displacement by excess rubidium. The isotope ^{42}K was used to measure the potassium so displaced into the external fluid.

In the determination, baker's yeast was suspended at a temperature of about 4–5° in 0·6 times its volume of 5% glucose containing 20 mM magnesium acetate. The magnesium ions here served to satisfy fixed anionic charges on the cell surface, and interfered very little with K^+ or Rb^+ on the carrier. (The temperature of 4–5° was chosen, as then K^+ uptake is considerably reduced but is still measurable.) KCl was now added to produce a similar suspension. Thirty seconds after addition of potassium, a very small volume of RbCl solution was added to produce a Rb^+ concentration external to the cells of about 10 m-equiv./l., and on shaking for a further half minute the external K concentration was measured. The increase found was due to displacement of potassium from the carrier. The mean of thirteen such experiments gave 0·09 m-equiv. K displaced per kg. yeast.

Since this method measured only the reduced form of the catalyst the total concentration was measured by bubbling hydrogen through the suspension to remove all the oxygen present and so completely reduce the carrier. The amount of potassium bound under these conditions was determined. The median value of six such experiments gave 0·12 m-equiv./kg. yeast. The iron content of yeast is about 0·60 m-equiv./kg. wet yeast, mostly present in the cell wall and the copper content about 0·06 m-equiv./kg. (Conway & Ryan, unpublished). Thus there is insufficient copper for binding of K but more than enough iron to act in the carrier enzyme.

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The Water Content of Rat Liver Mitochondria. By M. G. Harrington and Honor Geoghegan. (*Biochemistry Department, University College, Dublin*)

In studying the osmotic properties of isolated rat liver mitochondria several factors have been used for expressing the degree of swelling and/or the concentration of the inorganic ions present. These include the water content of the 'mitochondrial pad' (Fonnesu & Davies, 1955), values per gram fresh tissue, and values per litre of water in the fraction (McFarlane & Spencer, 1953). In an effort to assess the reliability of such reference properties, mitochondria have been prepared in sucrose solu-

tions of different molarities in the absence or presence of ethylenediaminetetra-acetate. The method of preparation used was a modification of that used by McFarlane & Spencer (1953). The washed mitochondrial pad was then analysed for total water, 'extra particulate' water and total nitrogen. The water contents of the mitochondrial pads varied within the accepted normal limits, while other properties showed large and apparently uncorrelated variations. The possible significance of these results will be discussed.

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Changes in Muscle Composition Associated with Increased Mg Levels. By D. Hingerty (introduced by E. J. Conway). (*Biochemistry Department, University College, Dublin*)

In general, changes in muscle potassium, whether increases or decreases, are paralleled by similar changes in magnesium content. After adrenalectomy, for example, an increase in muscle and plasma magnesium of adrenalectomized rats occurs (Conway & Hingerty, 1953); and increases in plasma magnesium after adrenalectomy were found by Harrop, Soffer, Elsworth & Tresher (1933) in dogs and by Zwemer & Sullivan (1934) in cats.

Besides the changes in K and Mg content in the muscle of adrenalectomized rats there was also observed (Conway & Hingerty, 1953) an increase in adenosinetriphosphate (ATP) and in phosphocreatine (PC), with a marked decrease in the hexose ester content of muscle tissue. Since magnesium ions have been shown to inhibit adenosine triphosphatase (Greville & Lehmann, 1943) it seemed possible that the changes after adrenalectomy mentioned above might arise from the changed levels of magnesium.

To test this hypothesis the magnesium levels of plasma and hence of muscle were directly raised by injecting $MgSO_4$ solutions intraperitoneally into rats.

Owing to the narcotic action of the Mg^{2+} ions such experiments could only be short-term. As a result of these injections the mean skeletal muscle content of magnesium was increased from $10{\cdot}6 \pm 0{\cdot}24$ (giving S.D. of mean) to $13{\cdot}1 \pm 0{\cdot}43$ m-mol./kg. Increases of ATP from $6{\cdot}5 \pm 0{\cdot}13$ to $7{\cdot}1 \pm 0{\cdot}19$ m-mol./kg. and of PC from $24{\cdot}7 \pm 0{\cdot}27$ to $26{\cdot}2 \pm 0{\cdot}62$ were observed. A fall in glucose 6-phosphate from $6{\cdot}08 \pm 0{\cdot}57$ to $3{\cdot}96 \pm 0{\cdot}44$ m-mol./kg. and an increase of glucose 1-phosphate from $3{\cdot}05 \pm 0{\cdot}21$ to $4{\cdot}16 \pm 0{\cdot}50$ m-mol./kg. also occurred, these various changes simulating effects after adrenalectomy. In one particular there was, however, a marked difference. The relatively small content of fructose 6-phosphate increased from $0{\cdot}92 \pm 0{\cdot}03$ to $1{\cdot}12 \pm 0{\cdot}08$, instead of falling to $0{\cdot}27 \pm 0{\cdot}02$ m-mol./kg. as occurs after adrenalectomy.

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Net Excretion of the Na ions from Na-rich Muscle and its Relation to the Redox Pump Theory. By Mary J. Carey and E. J. Conway. (*Biochemistry Department, University College, Dublin*)

Sartorii muscles of the frog (*Rana temporarii*) rendered Na-rich by immersion overnight in the cold in K-free Ringer–Conway fluid containing 104 mM-Na show no net excretion of Na ions when immersed at room temperature in similar fluid containing 104 mM-Na and 10 mM-K (Conway, 1946). Steinbach's experiments in this connexion are invalidated by a faulty statistical procedure (Conway, 1955, 1956).

When, however, the soaking overnight occurred in K-free Ringer–Conway fluid containing 120 mM-Na and the re-immersion took place in similar fluid containing 104 mM-Na and 10 mM-K, there was a considerable excretion of Na, as shown by Desmedt (1953) and confirmed by us in a large series of experiments. This excretion was greatly in excess of the small outward diffusion of Na ions from the interspace fluid.

We have studied this net excretion of Na^+ ions chiefly with respect to the action of inhibitors. The net excretion is practically completely inhibited by 2 mM cyanide, also by anoxia and by low temperature (0°), but is practically uninhibited by 2:4-dinitrophenol present even to the extent of 2 mM.

Comparisons of the oxygen consumption and the Na excretion shows that at the beginning or height

of the excretion of Na ions, the ratio Na^+ excretion/O_2 consumed approaches, but does not exceed, 4.

The extent of the data will appear from the following: Fifty-one experiments (306 muscles) carried out with equal Na^+ content in the K-free soaking fluid at 2–3° and in the re-immersion fluid with 10 mM-K, gave a value of $0{\cdot}3 \pm 1{\cdot}9$ m-equiv./kg. for the excretion, which is zero within the sampling error. With 120 mM-Na in the soaking fluid, fifty-five experiments (315 muscles) showed an excretion of $26{\cdot}9 \pm 1{\cdot}4$ m-equiv./kg. With the latter condition 2 mM cyanide gave an increase in Na of $6{\cdot}7 \pm 4{\cdot}2$ m-equiv./kg. (13 experiments, 66 muscles).

The fact that cyanide in small concentration (2 mM) anoxia, and low temperature, but not 2:4-dinitrophenol can practically completely inhibit the net excretion of Na indicates the operation of the cytochrome region of the respiratory chain, and that energy-rich bonds do not appear to be directly or indirectly involved. In short, the evidence accords very well with the redox pump theory.

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The Effect of Deoxycortisone Acetate and Cortisone on the Active Uptake of K and Excretion of Na from Red Corpuscles of the Rat. By L. O'Brien and E. J. Conway. (*Biochemistry Department, University College, Dublin*)

The effects on corpuscles from normal and adrenalectomized animals were examined. Losses of K and gains of Na were first produced by storage at 4° for 7 days, reverse changes being then produced by incubating at 37° with glucose, with and without the corticoids (1 mg. allowed per 5 ml. blood) (Harris, 1941; Danowski, 1941; Maisels, 1954). The Na and K content of the corpuscles were expressed per litre of the original corpuscles (before storage), allowance being made for volume changes. The results obtained may be summarized as follows:

Effects on K *uptake.* With corpuscles from the normal animal deoxycortisone acetate inhibited the uptake of K in the incubation stage with glucose (from a mean of 34·6–26·1 m-equiv./l. uptake of K; 4 experiments). A more marked inhibition is produced in corpuscles from the adrenalectomized animal (from a mean of 18·4 to −21·1 m-equiv./l. uptake of K; 5 experiments). Also with corpuscles obtained from adrenalectomized animals in which for 7 days after adrenalectomy 2·5 mg. of the hormone was injected daily, the inhibition is manifested in the incubation stage, without added hormone, and still further increased with such addition. The effects with cortisone were found to be similar in kind but were in general less marked.

Effect on Na *excretion.* For corpuscles from the normal animal the loss of Na in the incubation stage with DOCA was slightly increased (4 experiments). With corpuscles from the adrenalectomized animal, the gain of Na observed in the second stage (storage in the cold) increased again in the third (incubation) stage. With addition of the hormone the gain in this stage was much reduced. With corpuscles from adrenalectomized animals receiving 2·5 mg./day for 7 days after operation, a similar effect was obtained. The mean level of Na in these corpuscles before the cold storage was the lowest of the series (12·9 m-equiv./l.).

There was no indication that cortisone stimulated the excretion of Na. Its addition in the third stage with corpuscles from the normal animal had no effect in one experiment beyond the sampling error. With corpuscles from adrenalectomized rats receiving 2·5 mg. cortisone acetate daily for 7 days, the Na level in two experiments before storage were the highest in the series of experiments (23·5 m-equiv./l.). The Na levels increased during the third stage and still more after including the hormone, which contrasts with the effect of deoxycortisone acetate.

It will be seen from the above account that the effects of deoxycortisone acetate and cortisone acetate on the active transport of K ions across the membrane of the red corpuscles are very similar to those recorded for yeast (Conway & Hingerty, 1953). The effects on the transport of Na are not so well defined, but deoxycortisone acetate would appear to have in general some stimulating action, and this is not shown by cortisone.

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ACTH and Adrenal Ascorbic Acid in the Chick. By A. N. HOWARD and B. J. CONSTABLE. (*Dunn Nutritional Laboratory, Medical Research Council and University of Cambridge*)

The transient fall in adrenal ascorbic acid, such as follows the injection of adrenocorticotropic hormone (ACTH) in the rat or guinea pig, does not occur in the chick (Jailer & Boaz, 1950), quail (Zarrow & Baldini, 1952) or duckling (Zarrow & Zarrow, 1950). These authors found that the prolonged administration to birds of ACTH, or of certain stress agents, did nevertheless produce adrenal hypertrophy and hyperplasia; and they therefore concluded that the synthesis and release of adrenal corticoids was not dependent on a fall in concentration of adrenal ascorbic acid (which is low in birds).

We have found that single injections, of up to 10 i.u. lyophilized ACTH (Armour Laboratories), in 6-week-old chicks, produced no change in adrenal ascorbic acid concentration during the first 6 hr. after the injection. A 30% decrease in adrenal cholesterol concentration was observed at 6 hr. ($P<0.01$). Chicks fasted for 24 hr. were injected intramuscularly with five 1 hr. doses of 5 i.u. ACTH, and killed 1 hr. after the last injection. This produced a 20-fold increase in liver glycogen ($P<0.01$) and a 25% increase in blood glucose ($P<0.01$) compared with control animals, confirming the conclusion that ACTH can stimulate the production of the adrenal steroids affecting carbohydrate metabolism although having no effect on adrenal ascorbic acid.

When chicks were injected twice daily with 7·5 i.u. ACTH for 8 days the adrenal weight was increased by 30% ($P<0.01$) and adrenal cholesterol decreased by 60% ($P<0.001$). The adrenal ascorbic acid concentration was unchanged but whole blood and plasma concentrations decreased by 50% ($P<0.001$) and 60% ($P<0.001$). The reasons for this latter effect of ACTH on the blood are obscure: when single doses of ACTH were injected, no change in blood or plasma ascorbic acid occurred up to 12 hr. after injection.

With pituitary stimulants such as intramuscular injections of saline, insulin, histamine diphosphate, sodium salicylate (20 mg./100 g. body weight) no effect on adrenal ascorbic acid was observed, 2 hr. after injection; but a larger dose of salicylate (60 mg./100 g.) produced a 40% increase in plasma ascorbic acid ($P<0.01$) and a 20% depletion of adrenal ascorbic acid ($P<0.01$). This latter effect may be unconnected with synthesis of ACTH.

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Determination of Azide by Microdiffusion. By T. G. BRADY and JANE M. O'CALLAGHAN. (*Department of Biochemistry, University College, Cork*)

Azide ion, in the presence of strong acid, is converted into volatile hydrazoic acid, which can be absorbed by a silver nitrate solution, yielding insoluble silver azide and liberating free nitric acid. These reactions have been used as the basis for a sensitive and specific method for the determination of azide over a wide concentration range (10^{-2}–5×10^{-6}N).

0·1 ml. of $AgNO_3$ ranging from 0·1 to 0·001N, depending upon the azide concentration being estimated, and containing added indicator and Lissapol N is added to the central chamber of no. 2 Conway units. N-H_2SO_4 is placed in the outer chamber, and, after sealing with a paraffin mixture, the azide solution is added. Diffusion requires 2–2½ hr. at room temperature.

In the concentration range 10^{-2}–10^{-4}N azide, bromophenol blue in a buffered $AgNO_3$ solution is an efficient adsorption indicator with thiocyanate in the burette. At lower concentrations, the free nitric acid liberated is titrated with 2×10^{-4}N-NaOH using bromocresol purple as indicator.

Purification of Mammalian Adenosine Deaminase. By T. G. BRADY and W. O'CONNELL. (*Department of Biochemistry, University College, Cork*)

The small amount of alkaline phosphatase, persisting in extracts from the acetone fraction of intestinal mucosa containing the highest adenosine deaminase activity, has proved difficult to eliminate.

Electrophoresis on paper of such extracts shows four main protein bands, none of which is associated with deaminase or phosphatase activity. The deaminase and phosphatase activities are, however, present in approximately the same region on the paper, confirming the similar physical properties of these two enzymes.

In attempting to eliminate phosphatase, only limited success was achieved by refractionation with cold acetone followed by drying. On occasions, powders devoid of phosphatase activity have been obtained, but no substantial increase in deaminase activity occurred, although there was considerable loss in the total enzyme units recovered.

More success was achieved by ammonium sulphate fractionation followed by acetone fractionation. Starting with an extract having a specific activity of 450 for deaminase and 15 for phosphatase (enzyme units = μg. NH_3-N or μg. P liberated/minute at 37°) the first fraction, separating between 0 and 0·6 saturation, contained practically all the deaminase and phosphatase activity. After solution and dialysis, this preparation had an activity of 3500 for deaminase and 112 for phosphatase. Electrophoresis on paper gave two protein bands, one of which appears to be identical with one of the four protein bands in the original extract. The second band is not identical with any of the bands in the original extract.

Refractionation with ammonium sulphate gave a partial separation of the two enzymes, but fractionation with cold acetone gave more encouraging results. The bulk of the phosphatase was eliminated in the earlier fractions and the final fraction had a specific activity of 3900 for deaminase and 12 for phosphatase.

Initiation of Fermentation in Baker's Yeast Cells by Removal of Oxygen. By T. G. Brady, C. McGann and E. Tully. (*Department of Biochemistry, University College, Cork*)

Under normal conditions, baker's yeast is unable to ferment its reserve carbohydrate even when oxygen is absent (Stier & Stannard, 1936; Spiegelman & Nozawa, 1945). The addition of substances such as azide, 2:4-dinitrophenol and arsenite (Rothstein & Berke, 1952; Brady & Duggan, 1955) creates conditions in which fermentation of reserve carbohydrate does occur. The decrease in carbohydrate is accounted for by the quantitative production of alcohol and carbon dioxide (Brady & Duggan, 1955).

It has now been found that the establishment of anaerobic conditions alone, without the addition of any agents, leads to a smaller, but still definite fall in reserve carbohydrate. Alcohol and carbon dioxide are again produced, but in amounts much less than would be expected from the amount of reserve carbohydrate which has disappeared. When oxygen is readmitted to such a suspension, there is initially a stimulation in oxygen consumption. This initial burst in activity has also been noted by Stier & Stannard (1936) and Spiegelman & Nozawa (1945). Winzler & Baumberger (1938) noted a stimulated heat production in cell suspensions after periods of anaerobiosis.

The results are interpreted as indicating that yeast cells, as soon as respiration is suppressed, have a mechanism for mobilizing a portion of their reserve carbohydrate, and converting it into units of smaller size than hexose. The cells are thus prepared internally for their alternative mode of metabolism, i.e. fermentation. For fermentation to proceed, however, an exogenous supply of carbohydrate is necessary. When absent, the incipient fermentation ceases before the mobilized carbohydrate is used up. The transient stimulation of respiration which occurs on admission of oxygen is due probably to the rapid metabolism of the carbohydrate fragments already mobilized.

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A Urinary Chromogen Derived from Tea. By W. R. Fearon and W. A. Boggust. (*Department of Biochemistry, Trinity College, Dublin*)

The test is carried out by mixing 5 ml. of urine with 5 drops of a nickel reagent (0·1 M-$NiSO_4$ in 3 M-NH_4OH), and oxidizing by addition of 3 to 6 drops of 1 % (w/v) I_2 in KI. If the test be positive, a dark purple colour will develop and persist. The test is not given by the urine of non-tea-drinkers, including laboratory animals, but can be demonstrated in the urine of human subjects within 24 hr. of the ingestion of Indian, Ceylon, or China tea, and within an hour after the administration of 0·4 g. catechin. Gallic acid does not evoke a typical response. If Zn be substituted for Ni, a red pigment

is obtained, apparently identical with that formed in the elusive reaction observed by Godfried (1935). While many polyphenols and all tea infusions examined gave various colours when oxidized by I_2 in alkaline solution, none so far has been found to give the nickel reaction.

The urinary chromogen has been separated as a lead derivative and also by chromatography. It appears to be a phenol modified by conjugation with a carbohydrate residue.

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The Detection of Carbamyl Phosphate. By W. A. Boggust and W. R. Fearon. (*Department of Biochemistry, Trinity College, Dublin*)

The observation that carbamyl phosphate (CAP) can function as a carbamyl donor in biological systems (Jones, Spector & Lipmann, 1955, 1956) has led to a study of methods suitable for its detection and estimation. These include: (1) a selective colour reaction, and (2) transfer reactions to acceptors that yield carbamides capable of easy recognition. The only colour test so far obtained is an application of the benzidine-copper reaction, originally devised for the detection of cyanate (Fearon, 1926).

When 2 ml. of a dilute solution of CAP is added to a reagent prepared by mixing 5 drops of 5 % (w/v) benzidine in ethanol and 2 drops of 5 % (w/v) cupric acetate with 5 ml. of water, a brown-pink pigment forms, and gradually separates as a precipitate, insoluble in the commoner organic solvents. The test will detect 0·5 mg. of lithium CAP in 5 ml. of solution, and is negative with other Li salts, including phosphate, carbonate, bicarbonate, and carbamate. With cyanates, the pigment is a dark sepia-brown, and easily distinguishable from that given by CAP. Cyanide, bromide, iodide, thiocyanate and thiosulphate yield dark blue pigments with benzidine and Cu^{2+}, which can be extracted from solution. The test has been applied on paper chromatograms for the detection of CAP, using as reagent a 0·5 % (w/v) solution of benzidine in $CHCl_3$ containing 0·25 % cupric oleate.

Transfer reactions can be obtained, using hydrazine, phenylhydrazine, or *cyclo*hexylamine as carbamyl acceptor, and detecting the resulting carbamide by the diacetyl monoxime reaction (Fearon, 1939).

The semicarbazides obtained, respectively, from hydrazine and from phenylhydrazine have the advantage of not requiring an added oxidizer to generate the diacetyl pigment, while the pigment obtained from *cyclo*hexylcarbamide has the property of being soluble in $CHCl_3$, and thus recognizable. CAP required for the tests was prepared by the method of Jones, Spector & Lipmann (1955), in 45 % yield as the di-lithium salt. It was free from cyanate when tested by the pyridine-copper reaction (Werner, 1923).

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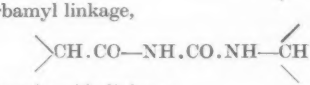
The Characterization of the Biological Carbamides. By W. R. Fearon and W. A. Boggust. (*Department of Biochemistry, Trinity College, Dublin*)

Naturally occurring compounds containing the monosubstituted-carbamide function, $R.NH.CO.NH_2$, include citrulline, allantoin, allantoic acid, carbamidosuccinic acid (carbamylaspartic acid), and α-carbamidoglutaric acid (carbamylglutamic acid). All these compounds give a typical red-purple carbamido-diacetyl reaction (Fearon, 1939), which is intensified by the presence of various modifiers, including tryptophan.

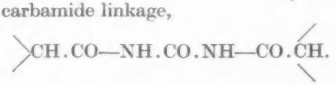
Many tissue extracts (muscle, liver, pancreas, yeast autolysate) and common proteins, such as the albumins of egg white and blood serum, caseinogen, fibrin, edestin, gluten and gelatin, give well-marked red-purple reactions, the significance of which is under investigation. As no compound other than a substituted carbamide has as yet been found to give the test, it is inferred that the chromogen is either a free carbamido side-chain, or

a carbamidogen, of which three types can be postulated:
(*a*) carbonyl linkage,

(*b*) carbamyl linkage,



and (*c*) carbamide linkage,



The free carbamide side-chain can be characterized by the fact that it gives a positive diacetyl reaction and a yellow *para*-dimethylaminobenzaldehyde reaction in N-H_2SO_4. No protein has yet been observed to give the tests under these conditions; the diacetyl reaction requires an acid concentration of about 5N-HCl or H_2SO_4, which suggests that a carbamide is being generated during the conditions of the test.

As regards the proteins, this cannot be attributed to desimidation of arginine units by the acid, since many arginine-containing proteins (protamines, insulin, crystalline trypsin and chymotrypsin) fail to give a carbamido diacetyl reaction; although, as we have found, citrulline can arise from arginine in yeast and bacterial autolysates. Of the possible carbamidogens present in proteins, the most plausible is the carbamyl linkage type, which is a tripeptide system, the middle unit of which is derived from carbamic acid (Martin & Synge, 1945). The course of the enzyme hydrolysis of caseinogen and the autolysis of baker's yeast has been followed chromatographically, and several copper complexes giving a positive diacetyl reaction have been obtained (Fearon & Boggust, 1954). The intensity of the diacetyl reaction remains approximately the same during the hydrolysis of caseinogen, but increases greatly during the autolysis of pancreas and yeast, which suggests the operation of a carbamyl assembly-transfer mechanism, or the opening-up of cyclic ureides.

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The Action of Leaf Ribonuclease. By R. Markham and J. L. Strominger. (*Agricultural Research Council Virus Research Unit, Molteno Institute, University of Cambridge*)

The leaf ribonuclease of Holden & Pirie (1955) has been investigated, and has been found to split all the internucleotide linkages of ribonucleic acids to yield nucleoside 2′:3′-phosphates. Of the latter the pyrimidine derivatives are apparently inert to further action of the enzyme, while the purine derivatives are hydrolysed slowly to give nucleoside 3′-phosphates exclusively. The purine cyclic nucleotides also act as donors in transesterification reactions involving nucleosides or nucleotides as acceptors.

Using the synthetic polymers of cytidylic acid, uridylic acid and adenylic acid as substrates it has been possible to investigate the activity of the enzyme on some of the types of linkage involved, and it has been found that polycytidylic acid is attacked at about 2% the rate at which polyuridylic acid is attacked under comparable conditions. Dinucleotides and trinucleotides are found in these digests, as in those from whole ribonucleic acid.

As an analytical tool, leaf ribonuclease may be used to detect terminal pyrimidine nucleoside 3′- (or 2′-) phosphate residues in polynucleotides, and it also provides an excellent method for the production of cyclic nucleotides, especially those containing pyrimidines, which may be obtained in quantitative yield.

Like pancreatic ribonuclease the leaf enzyme has the ability to build up polynucleotides if provided with the correct substrate, and this lends further support to the idea that ribonucleases may not normally be active as degradative enzymes, but may be concerned in the processes of synthesis and rearrangement of ribonucleic acids.

The leaf enzyme was made from pea plants and was provided by Mr N. W. Pirie, F.R.S., and Dr S. Pierpoint. The synthetic polynucleotides were made by the method of Grunberg-Manago & Ochoa (1955) and were given by Drs Ochoa and Heppel. Analyses were carried out according to Markham & Smith (1952) and Heppel, Whitfeld & Markham (1955).

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Pyridine Acetate Buffers for the Chromatography of Corticotropins on a Carboxylic Ion-Exchange Resin. By H. B. F. Dixon. (*Department of Biochemistry, University of Cambridge*)

Ion-exchange chromatography has been widely used for the purification and isolation of corticotropins (Dixon, Moore, Stack-Dunne & Young, 1951; White & Fierce, 1953; Li *et al.* 1954; Dixon & Stack-Dunne, 1955). Various methods have been used for obtaining the products in a dry, salt-free state, since cellophan allows the passage of corticotropins on dialysis. Hirs, Moore & Stein (1952) simplified the isolation of amino acids after separation by ion-exchange chromatography by using buffer solutions of ammonium formate and ammonium acetate, whose components were volatile.

Aqueous pyridine/acetic-acid systems have now been used as volatile eluents for chromatography of corticotropins and their derivatives on the carboxylic ion-exchange resin Amberlite IRC-50 (ground form XE-64), previously equilibrated with the eluent buffer. A solution of 15% (v/v) pyridine and 3·5% (v/v) acetic acid (pH 5·7) gave similar retention volumes for corticotropins to those given with the sodium phosphate buffer that Dixon & Stack-Dunne (1955) used with the same resin. This buffer (0·1 M-Na_2HPO_4, 0·1 M-NaH_2PO_4) had pH 6·7 and sodium-ion concentration 0·3 N. Similar resolution is obtained with the two systems, and the recovery (about 80%) judged by the ninhydrin reaction is comparable.

These observations agree with that of Dixon & Stack-Dunne (1955) that a solution of 30% pyridine and 4% acetic acid will elute corticotropins from a carboxylic resin, and do not conflict with the findings of Richter, Ayer, Bazemore, Brink & Folkers (1953) that with aqueous pyridine alone corticotropins remain on the resin. The pH is below those used previously for the chromatography of corticotropins, and the cation concentration has been raised to counteract the increase of retention volume produced by this lowering of pH.

Pyridine acetate was preferred (1) to ammonium acetate since it is more volatile and its buffering range is higher, (2) to collidine systems because of the limited miscibility of collidine with water, and (3) to ammonium bicarbonate because the more acid carbonate-bicarbonate buffers lose CO_2 to air and more alkaline conditions may cause slow breakdown of corticotropin A_1.

The pyridine used gave a negligible blank in the ninhydrin reaction of Moore & Stein (1954) after treatment at 50° by bromine (2 ml./l.) for 30 min. followed by redistillation (M. W. Rees, personal communication).

I am grateful to Miss M. B. Thomas for assistance.

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DEMONSTRATION

A Vibrating Table for Occasional Use with Microdiffusion Units and as a General Stirrer. By E. J. Conway. (*Biochemistry Department, University College, Dublin*)

Recently we had occasion to investigate the most rapid absorptions of ammonia and CO_2 in the standard no. 1 units (Conway & Dolan, 1954) and a vibrating table was designed which produced a striking shortening of the absorption time when 0·1–0·5 ml. of standard ammonium sulphate solution was used with 1·0 or 2·0 ml. of saturated K_2CO_3. The original model has been further improved, and is being demonstrated. This vibrating table consists of a circular wooden platform centrally supported by a metal sleeve, housing a compression spring and piston. A rod fixed to this sleeve is coupled through a ball-race to a cam eccentrically mounted on a shaft of a series-wound motor, the speed of which is adjustable by a rheostat. The use of magnetic pulses is also being investigated.

The effect of the vibrating table in reducing absorption times may be illustrated as follows:

When the outer chamber of a microdiffusion unit (no. 1; Conway, 1951) contains 0·2 ml. ammonium sulphate solution and 1·0 ml. saturated potassium carbonate the time for full absorption is reduced from about 60 min. to about 17 min., and with 0·5 ml. solution and 1·0 ml. saturated potassium carbonate it is reduced from about 122 to 24 min.

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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **355th Meeting** *of the Biochemical Society was held at the National Institute for Medical Research, Mill Hill, on Friday,* 12 *October* 1956, *starting at* 2.00 *p.m., when the following papers were read:*

COMMUNICATIONS

Serine and Inositol Phosphatides in Egg-yolk. By R. R. Dils and J. N. Hawthorne. (*Department of Medical Biochemistry and Pharmacology, Medical School, University of Birmingham*)

It is commonly assumed (e.g. Wittcoff, 1951) that phosphatidyl serine is absent in egg-yolk phosphatides, though Chargaff, Ziff & Rittenberg (1942) found 1·4 % amino acid N in those of lipo-vitellin. The presence of an inositol phosphatide having a phosphorus/inositol ratio of 1:2 has been reported by Malangeau (1955), using the solvent extraction procedure of Folch (1949). The present communication confirms the presence of small quantities of phosphatidyl serine and an inositol-containing phosphatide in egg-yolk lipids and describes their behaviour on silica columns prepared according to the method of Lea & Rhodes (1955).

The crude egg-lipids were prepared by freeze-drying the yolks, extracting with acetone and re-extracting the residue with chloroform-methanol (1:1, v/v). This chloroform-methanol extract was freed from sphingomyelin by repeated precipitations from ether with acetone, and from non-lipid contaminants by passage down a cellulose column (Lea & Rhodes, 1953). The product had an N/P ratio of 1·046.

Chromatographic separation of this phospholipid mixture on a silica column by elution with 10 % (v/v) methanol–chloroform yielded three distinct fractions. The first fraction closely followed the solvent front and contained serine, ethanolamine and inositol in roughly equal amounts. It corresponded to about 1·4 % of the total P eluted. The second fraction (phosphatidyl ethanolamine) was contaminated with about 3 % inositol phosphatide (assuming the P:inositol ratio of Malangeau). It contained traces of phosphatidyl serine. Using higher methanol concentrations for elution, these fractions will overlap, making the preparation of pure phosphatidyl ethanolamine more difficult. The third fraction (phosphatidyl choline) was obtained by continued elution and again contained inositol phosphatide.

For analysis, aliquots of the phosphatides corresponding to 30 μg. P were hydrolysed with 6N hydrochloric acid in sealed tubes for 16 hr. at 110° and applied directly to paper chromatograms. The solvent was *iso*-propanol–acetic acid–water (3:1:1, v/v/v).

Inositol phosphatide was also found in a sample of egg lecithin prepared as an acetone-insoluble and ethanol-soluble fraction purified on cellulose. Passage down an alumina column in 95 % ethanol (Hanahan, 1954) gave lecithin free from inositol, serine and ethanolamine.

We are indebted to the Medical Research Council for a grant to one of us (R. R. D.).

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Effects of Chondroitin Sulphate and Related Substances on Prostatic Acid Phosphatase. By Grace M. Jeffree. (*University of Bristol, Pathology Research Laboratory,* 18 *Guinea Street, Bristol,* 1)

Using high dilutions of seminal plasma in physiological saline, chondroitin sulphate partially protects prostatic acid phosphatase from surface denaturation, but never gives full activity. With protection by gelatine 0·05 % (w/v), chondroitin sulphate causes slight inhibition. Sodium alginate acts similarly, with less protection and greater inhibition. (Chondroitin sulphate, chain-length 165 residues, 0·5 % (w/v), 61 % protection, 9 % inhibition; sodium alginate, chain-length 556 resiues, 0·5 % (w/v), 28 % protection, 44 % inhibition.)

Hydrolysis of alginate to chains of 10–100 residues increases protection and decreases inhibition; further hydrolysis gives greater inhibition and

less protection. Hydrolysis of chondroitin sulphate to short-chain molecules increases inhibition and lessens protection. At like chain-lengths the two have approximately like effects.

Gluconate and saccharate inhibit the enzyme more markedly, with very slight protection. Inhibition is competitive with the substrate, and of an order similar to that of oxalate, shown by Tsuboi & Hudson (1955) to inhibit the enzyme competitively, though much less than tartrate.

Inhibition by sodium dodecyl sulphate is not competitive, but opposed to protection by gelatine and apparently due to surface denaturation of the enzyme, as found by Wills (1954) with other enzymes.

Inhibition by chondroitin sulphate and sodium alginate is partly competitive and partly due to enzyme denaturation.

Starch does not inhibit the enzyme, but gives some protection from surface denaturation. Starch hydrolysates show less protection and some inhibition. Glucose gives no protection, but in high concentrations shows inhibition; this is neither competitive nor due to surface denaturation, but associated with phosphate transfer from the substrate (*p*-nitrophenyl phosphate). Other hydroxyl compounds show inhibition with phosphate transfer (Green & Meyerhof, 1952; Bravermann & Chargaff, 1953). Long-chain $\alpha\omega$-glycols give some protection from surface denaturation. Hydroxyl compounds give much less protection than amino-compounds (Jeffree, 1955, 1956).

Competitive inhibition occurs with hydroxyl and carboxyl groups in similar configuration to that of tartrate (oxalate is an exception). Surface denaturation is associated with widely spaced acidic groups. Inhibition associated with phosphate transfer is found with non-acidic hydroxyl compounds.

The degree of polymerization of chondroitin sulphate alters its effect on prostatic acid phosphatase and so may affect the enzyme activity of metastases in bone from prostatic carcinoma.

This work was supported by a grant from the British Empire Cancer Campaign.

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The Isolation of 16α-Hydroxydehydro*epi*androsterone from the Urine of Normal Men. By K. Fotherby, A. Colàs, S. M. Atherden and G. F. Marrian. (*Department of Biochemistry, University of Edinburgh*)

Chloroform extracts of urine from normal men prepared after pretreatment of the urine by boiling at pH 7·0 for 1 hr. were shown by paper chromatography to contain a 'blue tetrazolium' reducing substance (X) which had R_F 0·48 in the system B_2 of Bush (1952). In the Girard reaction X became transformed to a substance Xa which appeared in the ketonic fraction and which had R_F 0·28 in the same solvent system. The transformation of X into Xa could also be effected by treatment with 0·1 N-H_2SO_4 for 2 hr. Further preliminary experiments using paper chromatographic methods showed that Xa, but not X, was digitonin-precipitable; and since the former gave a positive Pettenkofer reaction it seemed that it might be a steroid with the 5-en-3β-ol grouping and that X might be the corresponding 3:5-*cyclo*-6-hydroxy compound.

From a chloroform extract of a large volume of boiled urine Xa was isolated by a process involving chromatography of the neutral fraction on a magnesium silicate–Celite column using light petroleum containing increasing amounts of ethanol as eluant, Girard separation of the ketonic fraction, and partition chromatography on a Celite column using the system 80% (v/v) methanol in water/benzene. It had m.p. 176–180° (uncorr.) and $[\alpha]_D^{15}$ (ethanol) + 11°. Analysis for C and H indicated the probable formula $C_{19}H_{28}O_3$. It yielded a diacetate, m.p. 167–171° (uncorr.), $[\alpha]_D^{12}$ + 23° (ethanol).

On reduction in acetic acid solution with H_2 and a palladium black catalyst, Xa took up 0·97 molar equivalents of H_2, while using a platinum oxide catalyst it took up 1·94 molar equivalents. The fully reduced product yielded an acetate, m.p. 172–174·5° (uncorr.), and this melting point was not depressed after admixture with authentic androstane-3β:16α:17β-triol triacetate (m.p. 172–174·5°, uncorr.). Accordingly, Xa must be 17-oxoandrost-5-ene-3β:16α-diol (16α-hydroxydehydro*epi*androsterone).

By analogy with the 3-sulphate of 17-oxoandrost-5-ene-3β-ol (dehydro*epi*androsterone), which can be hydrolysed by heating in neutral aqueous solution to give 3:5-*cyclo*-17-oxoandrostan-6β-ol (cf. Lieber-

man, Mond & Smyles, 1954), it is concluded that the substance present in urine is the 3-sulphate of 16α-hydroxydehydro*epi*androsterone.

No X could be detected in extracts of control urines obtained from an adrenalectomized-ovariectomized woman, but after the administration of dehydro*epi*androsterone to this subject it was excreted in readily detectable amounts. Therefore 16α-hydroxydehydro*epi*androsterone is probably a metabolic product of dehydro*epi*androsterone and may have the same metabolic relationship to dehydro*epi*androsterone and androst-5-ene-3β:16α:17β-triol as 16α-hydroxyoestrone (Marrian, Loke, Watson & Panattoni, unpublished) may have to oestrone and oestriol.

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The Incorporation of ^{14}C-Aspartic Acid into the Proteins and Related Fractions in the Mouse Bearing the Ehrlich Ascites Carcinoma. By W. S. Reith. (*Medical Research Council Experimental Radiopathology Research Unit, Hammersmith Hospital, London, W.* 12)

Work chiefly performed *in vitro* on homogenates and on cell-free systems has led to the hypothesis (Hoagland, 1955, 1956; Borsook, 1955) that in the mechanism of protein biosynthesis the carboxyl groups of the amino acids are activated by adenosine triphosphate, and that these activated aminoacyl groups are bound through an adenosine monophosphate bridge to an enzyme acting as an intermediate amino-acyl transfer compound.

The following work is an attempt to find and investigate any labelled compounds of relatively low molecular weight which might be formed on administration of a ^{14}C-labelled amino acid to mice bearing the Ehrlich ascites carcinoma.

A solution of L-aspartic acid-^{14}C was injected intraperitoneally, and after a certain time interval the liver and the tumour cells were rapidly separated out, homogenized at low temperature with an equal volume of 0·66N perchloric acid, and the precipitates and the acid-soluble supernatants were studied separately by various methods. Both the liver and the tumour cell supernatants yield on paper electrophoresis four ^{14}C-containing zones moving to the positive pole, but there is a considerable difference between both supernatants in the strength and the electrophoretic mobility of one relatively slow moving zone. The ^{14}C-containing substances in the two fastest moving zones in both supernatants have been identified as uridylic acid-aspartic acid compounds in which all the ^{14}C-activity is contained in the aspartic acid moiety. The compound contained in the fastest zone differs from the one contained in the more slowly moving zone not only in electrophoretic mobility but also in the R_P values in various chromatographic solvent mixtures.

Each of the two zones contained also adenylic acid which was, however, devoid of ^{14}C-activity.

The supernatant from livers pooled from ten mice (15·6 g. fresh weight) contained in the fastest zone (L_4) a quantity of the uridylic acid-aspartic acid compound equivalent to 400 μg. of uridylic acid, while the slower zone (L_3) contained an equivalent of 2400 μg. The slower zone from the supernatant of the pooled tumour cells (C_3) contained an equivalent of 630 μg. of uridylic acid, corresponding to 198×10^{-9} μg. per cell.

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The *in vitro* Metabolism of Tissue Slices from Rats given Triethyltin Compounds. By Jill E. Cremer. (*Toxicology Research Unit, M.R.C. Laboratories, Carshalton, Surrey*)

Recently, Magee, Stoner & Barnes (1957) have shown that triethyltin hydroxide produces a specific lesion of the white matter of the brain and spinal cord in rats. The lesion appears to be an increase in extracellular fluid with no damage to the cells, and is reversible. No histological damage to any other tissues has been observed. Previous biochemical studies (Aldridge & Cremer, 1955), using a rat liver mitochondrial preparation, showed that triethyltin sulphate was a potent inhibitor of oxidative phosphorylation.

In the present study tissue slices have been used, and a method has been developed for estimating triethyltin. Tissue slice experiments have been carried out using the conventional Warburg technique with Kreb's glucose–Ringer–phosphate

as suspending medium. Measurements have been made of Q_{O_2}, pyruvic acid and lactic acid over a 75 min. period at 37°. Triethyltin was estimated by a modification of the method of Aldridge & Cremer (1956). *In vitro* results, using rat brain cortex, liver and kidney slices, showed that only brain slices were sensitive to the addition of triethyltin sulphate. The response was a lowered Q_{O_2} and pyruvic acid, and an increase in lactic acid. Brain slices were sensitive to concentrations of 8×10^{-7}M triethyltin sulphate added in the medium, from which slices took up 30% to give a concentration of approximately $1{\cdot}5 \times 10^{-5}$M in the total tissue water of the slices. Comparable concentrations of triethyltin were found in liver slices, although no biochemical effects were observed. Distribution of triethyltin in rats shows the highest levels to be in the red cells and liver, with a lower and even distribution throughout the other tissues examined, including brain.

Slices of tissues from rats on a diet containing triethyltin hydroxide have been examined. The metabolic response of brain slices from these animals was similar to that obtained in *in vitro* studies, while neither liver nor kidney slices differed from controls. After replacing rats on a normal triethyltin–free diet for 12 days no organotin could be detected in their tissues and a normal metabolic response was obtained for brain slices.

It has been shown that the metabolic response of normal tissue slices to triethyltin salts added *in vitro* can be duplicated in tissue slices taken from rats administered triethyltin salts.

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Biosynthesis of ^{14}C-labelled Uridine Diphosphoglucose (UDPG) and Uridine Diphosphoacetylglucosamine (UDPAG). By Evelyn E. B. Smith* and G. T. Mills. (*Department of Biochemistry, University of Glasgow*)

In view of the importance of uridine phosphoglycosyl compounds in metabolism, the following provides a convenient method for the preparation of UDPG and UDPAG in a ^{14}C-labelled form.

An acetate requiring mutant of *Neurospora crassa* (ac-3-sp) was grown at 30° for 4 days with vigorous aeration on *Neurospora* minimal medium (Beadle & Tatum, 1945) supplemented with acetate. A total of 5 l. medium was utilized containing 2·5 mc. [COOH-^{14}C]acetate, with a final concentration of 0·4% sodium acetate. 10 g. acetone powder of *Neurospora* was obtained at the end of this period.

5 g. acetone powder were extracted for 1 min. with 2×100 ml. portions boiling water, rapidly chilled, filtered, adjusted to pH 10 and applied to a Dowex 1 Cl column. UDPG and UDPAG were eluted with HCl and NaCl by the method of Cabib, Leloir & Cardini (1953), adsorbed on charcoal, eluted with 50% ethanol and the alcohol removed by distillation *in vacuo*. 40 μmoles pure UDPG and 50 μmoles UDPAG were obtained. Both materials were found to contain considerable radioactivity, the level being approximately 0·01 μc/μmole in both cases. Separation of the products of hydrolysis of these compounds by paper chromatography indicated that the base, pentose and hexose or hexosamine portions of the molecules were all labelled.

* Beit Memorial Research Fellow.

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Liver Mitochondria. By W. N. Aldridge. (*Medical Research Council Unit for Research in Toxicology, M.R.C. Laboratories, Carshalton, Surrey*)

A large variety of media are used for the study of the metabolic activities of mitochondria. In an attempt to rationalize the composition of the media, consideration has been given to the concentration of the major constituents of the cytoplasm of the liver cell. The aspects which have been considered are (*a*) the tonicity of the intracellular fluid, (*b*) the electrolytes, and (*c*) the pH of the cytoplasm.

A medium has been examined in which the potassium–magnesium–(phosphate + adenine nucleotide) ratio is approximately that in the cell. The properties of mitochondria will be discussed, particularly with reference to stimulation of oxidation by phosphate acceptors and oxidative phosphorylation.

The Isolation from Cod Liver of a Nucleotide Related to Adenylosuccinic Acid. By I. D. E. STOREY* and D. N. LOVE. (*Department of Surgery, University of Edinburgh*)

Carter & Cohen (1955) obtained from yeast autolysates an enzyme that catalysed a reversible reaction between adenosine 5′-phosphate and fumaric acid, the product being shown to have the structure 6-succinyladenosine 5′-phosphate ('adenylosuccinate'). In *Escherichia coli* extracts, adenylosuccinate is formed from inosine 5′-phosphate, L-aspartate and guanosine triphosphate (Lieberman, 1956).

Hitherto, the isolation of adenylosuccinate from natural sources has not been reported. Whilst attempting to isolate uridine-diphosphate-glucuronic acid from boiled cod liver extracts, we have obtained by barium fractionation and paper chromatography a nucleotide resembling adenylosuccinate in many respects. The nucleotide contains one molecular proportion of ribose and of total phosphate, when the molar extinction coefficient of Carter & Cohen (1955) is used. Acid-labile phosphate is absent. Hydrolysis with Russell's viper venom liberates all the phosphate as inorganic phosphate, and gives an ultraviolet-absorbing spot on chromatograms differing from adenosine.

The ultraviolet absorption spectra of the nucleotide in acid and alkaline solution also differ from those for adenosine 5′-phosphate, both maxima and minima lying at longer wavelengths, whereas they appear almost indistinguishable from those of adenylosuccinate (Carter & Cohen, 1955; Lieberman, 1956). Heating in dilute acid gave maxima at 275 mμ. in 0·25N-HCl and 273 mμ. in 0·25N-NaOH. The acid maximum is identical with that of the free purine from adenylosuccinate, and also of all the synthetic 6-substituted purines derived from aspartic acid, glutamic acid, cysteic acid, glycine and lysine. The first three named synthetic purines and that from adenylosuccinate were readily degraded by acid to a diazotizable amine (Carter, 1956). With our own material, the acidic maximum remained unchanged even after heating in 5·5N-HCl at 100° for 70 min. It seems likely, therefore, that the nucleotide differs from adenylosuccinate in the substituent at C-6.

Incubation of the nucleotide with a yeast autolysate causes the rapid appearance of an adenosine-type spectrum. A study of the reaction products should throw some light on the nature of the substituent group.

We thank Dr N. R. Jones for his kindness in collecting the cod livers, and for the provision of facilities for the preliminary extractions at the Torry Research Station, Aberdeen.

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* External Staff, Medical Research Council.

An Inositol-containing Phosphate in Liver Tissue. By J. N. HAWTHORNE and G. HÜBSCHER (introduced by R. R. DILS). (*Department of Medical Biochemistry and Pharmacology, University of Birmingham*)

Although free inositol (Schopfer, 1951) and inositol-containing phosphatides (Folch & LeBaron, 1956) are known to be present in different tissues of animals and also in plants, the occurrence of free phosphate esters of inositol has not so far been described. Fractionation of free acid-soluble phosphates of guinea-pig liver by precipitation with barium and lead acetate respectively, followed by chromatography on Nalcite SAR resin, yields a phosphate fraction containing inositol in significant amounts.

Fresh guinea-pig liver is homogenized in 10 vols. of 5% (w/v) trichloroacetic acid. The protein-free filtrate is treated with lead acetate at alkaline pH. The precipitate thus obtained is decomposed with H_2S, centrifuged and the supernatant is treated with barium acetate. The barium precipitate is discarded and the filtrate is reprecipitated with lead acetate. The Nalcite SAR resin (80–200 mesh, 0·8 × 12·0 cm. column) is prepared in the formate form. The fractions eluted with 0·03M ammonium formate containing 0·01N formic acid are combined and concentrated *in vacuo*.

The inositol ester has not yet been obtained completely pure but its electrophoresis in borate buffer (Foster, 1953) suggests that it is the monophosphate. Its behaviour on the anion-exchange column mentioned is similar to that of the inositol phosphate released on mild alkaline hydrolysis of a crude liver inositide fraction (McKibbin, 1954; Hawthorne, 1955) which has been shown to contain inositol monophosphate (McKibbin, 1956). Paper chromatography in 6:3:1 (v/v/v) propanol–ammonia–water gives for the inositol phosphate an R_F value corresponding to that obtained with inositol monophosphate prepared from phytic acid.

Hydrolysis of the inositol ester in a small quantity of 6N-HCl in a sealed tube and chromatography of the hydrolysate in 3:1:1 (v/v/v) *iso*propanol–acetic acid–water showed the presence of marked amounts of inositol, small quantities of serine and a trace of ethanolamine. No choline or glycerol were detected.

The inositol phosphate present in liver tissue might be an intermediate in the biosynthesis of phospho-inositides, analogous to phosphorylcholine in lecithin biosynthesis.

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The Nature of the Gamma Globulins in Lymphadenoid Goitre. By I. M. Roitt, Deborah Doniach and P. N. Campbell. (*Institute of Clinical Research and Courtauld Institute of Biochemistry, Middlesex Hospital, London, W.* 1)

Lymphadenoid goitre (Hashimoto's disease) is a chronic disease of the thyroid often asociated with myxoedema. It is characterized by widespread infiltration of the thyroid gland by lymphoid tissue containing a large proportion of plasma cells. The thyroid follicles are disrupted, the colloid gradually disappears and the follicle cells undergo metaplasia.

The serum of patients with Hashimoto goitre contains a high concentration of γ-globulins which produce abnormalities in the flocculation tests of liver function (Fromm, Lascano, Bur & Escalante, 1953; Luxton & Cooke, 1956; Skillern *et al.* 1956). The abnormal findings in the serum gradually disappear about 6 months after surgical removal of the goitre (Doniach & Vaughan Hudson, 1956).

In order to examine the nature of these raised γ-globulins, the sera of patients with Hashimoto goitre were fractionated by zone electrophoresis on cellulose columns (Flodin & Porath, 1954) under the conditions described by Campbell & Stone (1956). The serum (1·0 ml.) was run for 40 hr. with a current of 10 mA on a column of cellulose (45 cm. × 1·5 cm.). Under these conditions, the albumin migrated off the column. In most cases the γ-globulins were resolved into two fractions. However, no clear-cut qualitative differences between the γ-globulins in Hashimoto and in normal serum could be detected by this method.

Hashimoto serum gave precipitin reactions with saline extracts of thinly sliced human thyrotoxic thyroid. Serum from normals and from patients with thyrotoxicosis and multiple myeloma failed to give any precipitate under the same conditions. This suggests that the raised γ-globulins in Hashimoto goitre may reflect an auto-antibody response to potential antigens contained in the diseased thyroid.

We wish to thank Mr R. Vaughan Hudson, F.R.C.S., who allowed us to study his patients, for his great interest in this investigation.

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The Preparation of [16-^{3}H]Progesterone. By W. H. Pearlman. (*Guy's Hospital Medical School, London, S.E.* 1)

Radioactive progesterone of a very high order of specific activity was required for metabolism studies. It seemed likely that such a product might be prepared with comparative ease and economy by catalytic reduction of a suitable unsaturated steroid with tritium. For this purpose 3β-hydroxypregna-5:16-dien-20-one 3-acetate (I) was selected as starting material. This underwent reduction at position 16:17 (but not at 5:6) in cyclohexane in a tritium-hydrogen atmosphere with '10%' palladium-charcoal as catalyst; an 88% yield of [16:17-^{3}H]3β-hydroxypregn-5-en-20-one 3-acetate (II) was obtained representing a 20% radiochemical yield. The tritium at C-17 is labile and was removed after equilibration of (II) with alkali to furnish [16-^{3}H]3β-hydroxypregn-5-en-20-one (III). The

latter (III) was converted on Oppenauer oxidation to [16-^{3}H]progesterone (IV). It is possible that compounds (II), (III) and (IV) also contain a small amount of stably bound tritium at C-15.

Two runs (I → IV) were made starting with: (*a*) 1·41 m-mole of (I) and 0·1c of ^{3}H, and (*b*) 0·47 m-mole of (I) and 2·0c of ^{3}H. The specific activities of the final product (IV) were 0·09 and 0·5c per m-mole respectively. These preparations of radioactive progesterone (IV) have proved useful in the author's experience (unpublished experiments) in studying those intermediary metabolic reactions of the hormone not involving the loss of the C-17 side chain; in the latter event, the isotope would probably be lost.

D-Glucose in the Blood of the Domestic Fowl. By D. J. BELL (introduced by W. M. MCINDOE). (*Agricultural Research Council, Poultry Research Centre, Edinburgh* 9)

Paper electrophoresis of concentrated, deproteinized fowl blood showed glucose as the sole detectable sugar. 1:2-5:6-Di-*O*-*iso*propylidene-D-glucofuranose was isolated by Bell's (1947) method.

The results of colorimetric determinations using the stabilized benzidine reagent of Jones & Pridham (1954) agreed with D-glucose determinations using glucose oxidase (Keilin & Hartree, 1948). The values were somewhat lower than, though of the same order as, those obtained by many previous workers using mainly the Folin–Wu and Hagedorn–Jensen methods. Somogyi's (1952) cuprimetric method applied to filtrates got by both $Ba(OH)_2$—$ZnSO_4$ and NaOH—$CdSO_4$ treatments gave somewhat high values. Resin deionization (Strange, Dark & Ness, 1955) of filtrates got by Hunter's (1956) method, gave solutions where both benzidine and copper methods were in agreement.

In whole bloods, hens have a higher average glucose level than cocks: hens (4–6 months) 216; (24–25 months) 202; (36–60 months) 187; cocks (4–6 months) 201; (12–24 months) 158. (Lower values were found in fasted animals.) These differences are probably due to differences in erythrocyte volume; fowl erythrocytes were found to contain little or no glucose.

The benzidine method (cf. Huggett, 1956) is simple and reliable; the colour is stable for at least 24 hr. It can be used on filtrates made according to Hunter (1956) or with trichloroacetic acid.

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Turnover of Protein in *Escherichia coli*. By J. MANDELSTAM. (*National Institute for Medical Research, Mill Hill, London, N.W.* 7)

Suspensions of *Escherichia coli* can synthesize some adaptive enzymes even when starved of nitrogen (Wainwright & Nevill, 1956; Mandelstam, 1956). The source of the material used for such synthesis was puzzling in view of reports that the bacteria do not contain (protein) precursors of enzymes, nor do their proteins break down (Hogness, Cohn & Monod, 1955), nor have they any detectable free amino acid pool (Taylor, 1947).

Analysis, by paper chromatography, of hot water extracts of bacteria grown in glucose and ammonium salts revealed a free amino acid pool varying from 0·3 to 1% of the dry weight depending upon bacterial strain and the phase of growth. The following amino acids have been identified: glycine, alanine, glutamic acid, aspartic acid, arginine, lysine, valine, phenylalanine, tyrosine, leucine and isoleucine.

During nitrogen starvation the free amino acid pool slowly increases, and the following experiments support the view that the amino acids thus released are derived from breakdown of protein. A leucine-requiring strain of bacteria was grown with [1-^{14}C]-leucine, washed to remove residual leucine, and then subjected to nitrogen starvation for 2 hr. The free amino acids increased from 0·23 to 0·48% of the dry weight. The leucine liberated (one-sixteenth of the total) had the same specific activity as the leucine originally used. These figures indicate a net rate of protein breakdown of 0·5% in 2 hr. The gross rate of breakdown may be a good deal higher because other experiments showed incorporation of

radioactivity into protein by washed suspensions, and this was inhibited by chloramphenicol (20 μg./ml.). There is thus evidence of both breakdown and synthesis of protein in such suspensions.

The turnover rate is negligible in terms of a rapidly growing culture but is more than sufficient to provide material for the formation of individual adaptive enzymes in non-growing suspensions.

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The Fractionation of Bovine γ-Globulin by Partition Chromatography. By ELIZABETH M. PRESS and R. R. PORTER. (*National Institute for Medical Research, Mill Hill, London, N.W. 7*)

In earlier papers (Porter, 1955; Humphrey & Porter, 1956; Askonas, Humphrey & Porter, 1956) a technique has been described for fractionating rabbit γ-globulin by partition chromatography. It has been shown that some fractionation of antibodies could be achieved, and evidence was obtained to suggest that the complexity of γ-globulin arises from different cells producing slightly different kinds of molecule. An attempt has now been made to see if a similar method would separate fractions which differed chemically from each other. The *N*-terminal amino acid was chosen as the easiest chemical characteristic to follow and γ-globulins from human and bovine serum were used, as they have a variety of terminal acids in contrast to rabbit γ-globulin which has only one. It was found that the γ-globulin from these three species, which are very similar, in most chemical and physical properties, differ very greatly in their stability, solubility and partition coefficients in the two liquid phases produced by mixtures of phosphate buffers and glycol ethers. No system suitable for use with human γ-globulin was found, but bovine γ-globulin could be fractionated by partition between the phases produced by mixing 2120 g. phosphate buffer 2·5M pH 9·0, 2672 g. 10% (w/v) aqueous urea and 548 g. diethyl carbitol at 6·0 ± 0·1°. A broadly spread peak, somewhat similar to that found using rabbit γ-globulin, was obtained and individual fractions when re-run appeared with distinct characteristic R values, suggesting that genuine fractionation was occurring. When this broadly spread peak was divided into 5 fractions and assayed for *N*-terminal acids, it was found that the principal terminal acid, valine, and also glutamic acid, were present in almost equal amounts in all fractions, while alanine was present only in the faster running fractions, and the content of aspartic acid and serine also varied significantly. The low total content of *N*-terminal acids in the original material, and even lower content of some of the fractions, suggests that either some terminal amino groups are unable to react with 1-fluoro-2:4-dinitrobenzene, or a significant proportion of bovine γ-globulins exist as cyclic molecules.

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Fluorimetric Estimation of Intermediary Metabolites. By P. GREENGARD.* (*National Institute for Medical Research, Mill Hill, London, N.W. 7*)

Oxidized and reduced pyridine nucleotides differ not only in their ultraviolet absorption properties but in their fluorescence properties as well. The difference in fluorescence of the oxidized and reduced forms has been used as the basis of a method for the estimation of a variety of intermediary metabolites which could be enzymically coupled either to the oxidation or to the reduction of either diphosphopyridine nucleotide or triphosphopyridine nucleotide. The method is about 100 times more sensitive than the corresponding procedures based upon ultraviolet absorption. Using fluorescence measurements, pyruvic acid, phosphopyruvic acid, adenosine diphosphate, adenosine triphosphate and glucose 6-phosphate (Greengard, 1956), as well as α-ketoglutarate and *iso*citrate, were estimated in amounts as low as 2×10^{-10} mole. A further tenfold increase in sensitivity was obtained by converting the oxidized pyridine nucleotides into highly fluorescent derivatives. This was achieved by the established procedure of condensation with methyl ethyl ketone in an alkaline medium.

Glucose 6-phosphate and adenosine triphosphate were determined using the combined hexokinase-glucose 6-phosphate dehydrogenase system from yeast (Kornberg, 1950). Glucose 6-phosphate de-

* Doris Duke Foundation Fellow of the National Paraplegia Foundation, U.S.A.

hydrogenase was found not to require the addition of Mg^{2+} ions. Even when ethylenediaminetetra-acetate was added in order to chelate possible traces of Mg^{2+}, the glucose 6-phosphate dehydrogenase was still fully active and, since hexokinase, as is well known, is a Mg^{2+}-requiring enzyme, it was possible to make separate estimations of glucose 6-phosphate and adenosine triphosphate in a mixture of the two.

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Spectrophotometric Evidence for the Presence of Pyridine Nucleotide in Heart Muscle Sarcosomes. By F. A. Holton. (*Medical Research Council Experimental Radiopathology Research Unit, Hammersmith Hospital, London, W.* 12)

In previous work it was shown that rat heart sarcosomes contain at least 6 μmoles of diphosphopyridine nucleotide (DPN) per g. protein, but their difference spectrum (anaerobic minus aerobic steady states) did not include the expected maximum in the region 320–350 mμ. when the reducing substrate was β-hydroxybutyrate (Holton, 1955). These spectral data have been quoted recently by Chance (1956) as evidence that the sarcosomes used in this and other work contained little pyridine nucleotide. The direct estimation of DPN quoted above does not agree with this view, and recent experiments suggest why the absorption maximum was not observed in the earlier work. A prominent peak at 320 mμ. has now been successfully demonstrated in the difference spectra of both rat and rabbit heart sarcosomes.

Heart sarcosomes were suspended in a 0·32 osmolar mixture containing DL-β-hydroxybutyrate, adenosinetriphosphate (ATP), ethylenediaminetetra-acetate and sucrose. The suspension was contained in an illuminated optical cell, and could be aerated by means of a pumped circulation while under continuous observation. On stopping the pump, the oxidase activity of the sarcosomes rapidly exhausted the dissolved oxygen and the suspension became anaerobic. The accompanying changes of extinction corresponding to reduction of respiratory pigments were measured at a bandwidth of 4–5 mμ.

The difference spectra obtained by repeating the aeration and deoxygenation cycle at different wavelengths from 300 to 445 mμ. showed a marked peak at about 320 mμ. This peak was probably caused mainly by the oxidation and reduction of the bound DPN of the sarcosomes. Its magnitude was approximately half that of the simultaneous extinction change at 445 mμ. and corresponded to 50–60 % of the extinction change which would have occurred at 340 mμ. if all the bound DPN had been free in clear solution and had been fully oxidized in the aerobic steady state and fully reduced in the anaerobic steady state.

In the earlier work quoted above measurements were not carried out below 320 mμ. and so the peak of the absorption band was not detected. However, positive changes of extinction at 320–340 mμ. were reported there. The present experiments support the conclusion then arrived at, that these changes represented reactions of endogenous DPN, and oppose Chance's (1956) conclusion that the sarcosome preparation of Slater & Holton (1953) possesses little pyridine nucleotide.

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DEMONSTRATIONS

A Two-Dimensional Paper Chromatogram Scanner. By E. A. Piper and H. R. V. Arnstein. (*National Institute for Medical Research, Mill Hill, London, N.W.* 7)

The apparatus records the amount of radioactive material on a paper chromatogram 40 cm. × 40 cm. The sheet is scanned in centimetre squares from left to right in 40 lines; each square centimetre is counted for 1 min., a chromatogram taking about 27 hr. for complete scanning. The radioactive count is drawn on a pen recorder as a series of lines whose amplitude is proportional to the activity. When the whole of the chromatogram has been scanned, the apparatus returns to the starting position and switches itself off. The apparatus consists of a vertical frame-work in which the paper chromatogram is moved horizontally and vertically by motors whose operation is controlled by a relay unit which is housed in a Post Office bench rack, together with the apparatus for receiving the pulses and driving the pen recorder.

A Windowless Gas Flow Counter with Automatic Recording. By E. A. PIPER. (*National Institute for Medical Research, Mill Hill, London, N.W. 7*)

Equipment used in the Quantitative Analysis of Protein Hydrolysates by the Moore and Stein Method. By S. JACOBS. (*National Institute for Medical Research, Mill Hill, London, N.W. 7*)

The Catabolism of Certain Plasma Proteins Studied by Means of the Isolated Perfused Rat Liver. By A. H. GORDON. (*National Institute for Medical Research, Mill Hill, London, N.W. 7*)

The behaviour of plasma proteins trace-labelled with ^{131}I by the method of McFarlane (1956) has been studied in the isolated perfused rat liver. Under these conditions and for periods of up to 5 hr. the amounts of protein broken down (measured as ^{131}I rendered non-precipitable by 8% trichloroacetic acid) has been found to vary very widely. Apparently the liver is able to remove very rapidly traces of plasma proteins which have become modified in certain ways. In an attempt to elucidate this behaviour, plasma protein preparations isolated by electrophoresis and by precipitation with salt and ether have been investigated. In addition, the effect of preliminary denaturation and aggregation of the labelled protein have been examined. The great importance of this latter factor was shown in a perfusion using blood from rats previously immunized against human serum albumin. On addition to the perfusate, this protein was rapidly taken up by the liver and already after 250 min. 16·3% had been broken down. On the other hand, human albumin which had been denatured with 6M urea and then regenerated (Bernheim, Cooper & Erickson, 1942) was broken down only twice as fast as before treatment. To attempt to discover the *in vivo* rate of catabolism by the liver of the unmodified plasma proteins and thus to provide a base-line for the above results, several perfusions have been carried out with the addition of plasma from rats which, 2 days previously, had received the ^{131}I protein by intraperitoneal injection. Very low rates of breakdown were thus obtained (0·23–0·29% of added rat albumin and 0·54% of the γ-globulin broken down in the 5 hr. period). Furthermore, no difference could be detected between the rate of breakdown of rat and human albumins. If the amount of blood in the perfusion circuit be taken into account, the observed low rates of catabolism, at least of rat plasma albumin, would appear to suggest that *in vivo* the liver can only be responsible for approximately 10% of the total breakdown of this protein.

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A Fluorimeter Suitable for the Estimation of Intermediary Metabolites. By P. GREENGARD and C. PAYNE. (*National Institute for Medical Research, Mill Hill, London, N.W. 7*)

The fluorimeter, which is simple in construction, combines high sensitivity with a low optical blank. Exciting light from a 125w MB/D mercury vapour lamp is focused at the centre of the cuvette. The cuvettes are Pyrex test tubes (75 × 10 mm.), selected from ordinary laboratory stock on the basis of fluorescence measurements. An EMI end-window photomultiplier (type 6097B) is used as the detector of fluorescent light. This type of photomultiplier has a greater sensitivity than the type commonly used in recent fluorimeters. Further, since the large diameter (1·5 in.) photocathode is a more efficient collector of light, the need for focusing lenses on the secondary side was avoided by suitable positioning of the photomultiplier.

The use of the fluorimeter will be illustrated by the enzymic estimation of adenosine diphosphate and α-ketoglutarate.